# FROM ECOLOGY TO CANCER BIOLOGY AND BACK AGAIN

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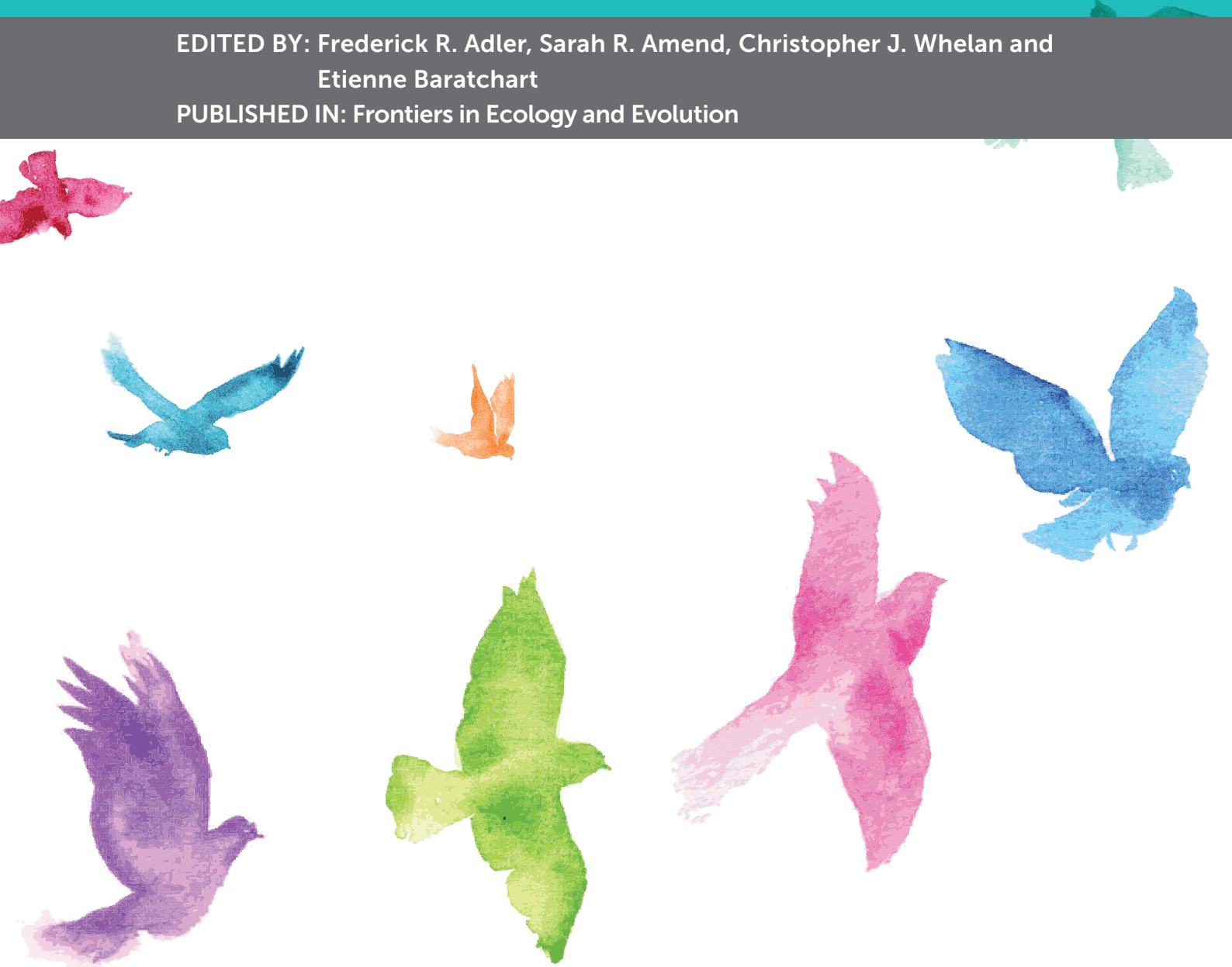
PUBLISHED IN: Frontiers in Ecology and Evolution



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ISSN 1664-8714
ISBN 978-2-88974-615-6
DOI 10.3389/978-2-88974-615-6

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# FROM ECOLOGY TO CANCER BIOLOGY AND BACK AGAIN

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**Citation:** Adler, F. R., Amend, S. R., Whelan, C. J., Baratchart, E., eds. (2022). From Ecology to Cancer Biology and Back Again. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-615-6

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frontiers in Ecology and Evolution

EDITORIAL
published: 10 February 2022
doi: 10.3389/fevo.2022.840375

# Editorial: From Ecology to Cancer Biology and Back Again

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**Keywords: cancer, ecology, competition, community ecology, metastasis, evolution**

**Editorial on the Research Topic**

**From Ecology to Cancer Biology and Back Again**

Application of evolutionary concepts to the study of cancer progression transformed cancer research in the 1970's (Cairns, 1975; Nowell, 1976) and inspired novel approaches to therapy. This work provided the framework for foundational discoveries in cancer genetics including those related to tumor cell heterogeneity, inherited risk of cancer, and synthetic lethality. Of the four classically defined forces of evolution (mutation, gene flow, genetic drift, and selection), however, only mutation is firmly classified in the field of genetics. The remaining three are ecological: gene flow depends on the movement of individuals, genetic drift on how population sizes vary in time and space, and selection on interactions with the biotic and abiotic environment.

Researchers are increasingly applying the ecological principles that underlie evolution to study cancer biology, appreciating that understanding the complex ecology of the tumor is essential to successfully treat this lethal disease. Conversely, the conceptual framework and quantitative tools from cancer biology have the potential to transform the understanding of the complexity of ecology itself, opening new ways to address the ecological challenges that define our times.

This Research Topic, therefore, was curated to support two goals.

1) Integrate ecosystem, behavioral, and physiological ecology into the study of cancer. The evolution that leads to resistance and metastasis is driven not only by the heterogeneity and phenotypic interactions that are the purview of ecology focused on the species level, but also by the flows of energy, materials and nutrients, and the changing phenotypes of individuals.
2) Use the insights of cancer ecology to rethink ecology itself, in particular by using modern molecular and genetic tools to address core questions and to apply them to forecasting and restoration.

## FROM THE CORE ISSUES IN ECOLOGY TO CANCER AND BACK AGAIN

Many of the papers in this Research Topic focus on the core issues that have defined the science of ecology since its founding: persistence of species, the maintenance of diversity and coexistence, and the distribution and abundance of species and their interactions.

Interactions among cancer cells and those between cancer cells and host cells display many ecological processes, including niche construction, resource exploitation (Huntly et al.; Kareva and Brown; Somarelli; Wu), predator-prey interactions (Peplinski et al.; Somarelli), source-sink population dynamics (Cunningham et al.), game-theoretic interactions among different cancer cell types (Kareva and Brown; Noble et al.; Pressley et al.), and hijacking of signaling mechanisms, including those governing metabolic pathways and immunological defenses (Bukkuri and Adler).



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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *21 December 2021*
***Accepted:*** *05 January 2022*
***Published:*** *10 February 2022*

***Citation:***
*Adler FR, Amend SR, Baratchart E and Whelan CJ (2022) Editorial: From Ecology to Cancer Biology and Back Again. Front. Ecol. Evol. 10:840375. doi: 10.3389/fevo.2022.840375*

These interactions often involve the tradeoffs that support coexistence of different tumor cell types (Huntly et al.; Kareva and Brown). In relation to ecological management, control of tumors parallels the control of invasive species (Neinavaie et al.) and combinations of approaches may be designed to drive the extinction of cancer (Gregg).

## FROM PHENOTYPES TO ECOLOGY AND BACK AGAIN

Advancing technological methodology has enabled the identification of individual cell phenotypes, including physiological states that determine cell behaviors and interactions among cancer cells and between cancer cells and the tumor microenvironment. This concept underpins tumor microenvironment research, and highlights the power of ecological concepts to understand tumor biology, progression, and lethality (Bissell and Hines, 2011; Myers et al., 2020). In ecology, dormancy plays a key role in the life histories of organisms that deal with unpredictable environments, and this phenotypic state is often observed clinically prior to cancer recurrence (Kostecka et al.; Miller et al.). While these similarities are informative, there are key differences between cancer cell and animal dormancy, with cancer cells often altering rather than simply slowing metabolism. These reversible metabolic changes may help cancers survive fluctuating resources and promote expensive but flexible glycolysis over or simultaneous with oxidative phosphorylation (Huntly et al.). These metabolic differences reveal themselves in scaling relationships between size and metabolism, with tumors showing strong and consistent scaling relationships that differ from healthy tissue, such as a high volumetric scaling factor that reflects the high resource needs of tumors (Brummer and Savage). These changes in metabolism can interact with ecological factors to alter the foraging behavior and growth of mice with cancer (Makin et al.). Differences in cell phenotypes can generate novel interactions. For example, one cancer cell population can exploit another one, increasing its fitness to the detriment of the other (Noble et al.).

## FROM ECOLOGY TO EVOLUTION AND BACK AGAIN

Despite the underlying stochasticity of mutation, some aspects of cancer are quite predictable, with cancers showing convergent evolution on the hallmarks of cancer by quite different mechanisms due to selection driven by the ecology of the cancer (Somarelli). On the other hand, predicting cancer evolutionary dynamics at the patient level remains quite challenging. Although numerous genome sequencing studies have offered some understanding of the cancer clonal makeup, the lack of phenotypic characterization of the tumor composition can make interpretation difficult (Plutynski). The ecological factors that drive cancer evolution can provide the crucial bridge. As an example, the rapid evolution induced in human-dominated ecosystems by harvesting and habitat change parallels the selection placed on cancer by treatment, generating the full range of qualitative and quantitative resistance (Pressley et al.). Resource availability also creates strong selection, with evidence supporting roles of high availability of glucose, iron, or phosphate promoting aggressive cancer growth (Wu). Cancer cells are well-known to tolerate low oxygen levels, and this potential may reflect the role of hypoxia in shaping cell differentiation during development, and argue that cancer cells might face challenges in overcoming the evolutionary legacy to retain stem like characteristics during normoxia (Carroll et al.).

Studies of evolution often focus on mutations. However, polyploidization and chromosomal instability lead to the accession of the polyaneuploid cancer cell (PACC) state that enables dormancy, treatment escape, and relapse initiation (Kostecka et al.). Changes in cell motility and collective behavior underlie metastasis, the deadliest phenotype of solid tumors. Parallels between metastasis and invasion in ecology may elucidate how metastatic cells colonize and adapt to foreign soil (Neinavaie et al.), pointing a way to understand and predict their spread.

## FROM EVOLUTION TO THE PREVENTION AND TREATMENT OF CANCER AND BACK AGAIN

Application of ecological and evolutionary principles has the potential to directly transform cancer patient care in diagnostics (Maley et al., 2017) and novel treatment strategies (Pienta et al., 2008; Amend and Pienta, 2015; Whelan and Gatenby, 2020). Indeed, many successful anti-cancer treatment strategies (e.g., bone marrow transplant in leukemias, immunotherapy checkpoint inhibitors in many solid tumors) are truly tumor ecology-informed therapeutic strategies. Specific application of an eco-evolutionary framework will identify other avenues for treatment innovation. Elucidating how metabolic needs shape cancer evolution implicates patient diet and lifestyle in cancer risk (Wu) and suggests dietary therapies that could complement chemotherapy and other conventional treatments (Gregg). Eco-evolutionary principles open up new therapeutic opportunities based on competition (Pressley et al.; Somarelli), dormancy (Kostecka et al.), predation (Peplinski et al.), and positive cell interactions (Noble et al.).

## FROM QUESTIONS TO CONCLUSIONS AND BACK AGAIN

Several themes and challenges emerge from this collection. First, the study of cancer cells in a tumor gives new insight into the regulation of healthy systems (Bukkuri and Adler). The cancer cells in a tumor demand and use resources like individuals in ecology (Cunningham et al.; Wu), and those resources include signaling molecules like hormones that mirror signals between individuals as in populations of plants (Kareva and Brown). Placing cancers in the larger biological context of evolution and development makes sense of properties like tolerance of hypoxia (Carroll et al.). Like species, cancer cells can, at least hypothetically, go extinct well-before their hosts (Gregg).

Second, heterogeneity matters. Individuals, cells, locations, and times differ (Huntly et al.), and these differences feedback

to affect each other (Gregg). The mechanisms underlying heterogeneity, can be genetic and heritable (Neinavaie et al.) or plastic and epigenetic (Gregg). Third, evolution and ecology are intimately linked. As in nature, evolution in cancer is driven by ecological interactions among cancer cells and between cancer cells and cells of the host, including fibroblasts, immune cells, the associated vasculature, and many other components of the tumor microenvironment.

The levels of selection created by population sub-structuring are critical from the perspective of the patient and the treating oncologist. For example, the success of temporal escape through dormancy depends on the behaviors of the individual cancer cells (Kostecka et al.; Miller et al.). Rates of evolution determine the ability to respond to novel selection regimes, and, in addition to mutation rates, these rates emerge from population dynamics (Pressley et al.). The efficiency of selection depends on population size and population structure. As in ecology, it is important to consider that observed traits may not be adaptive, but rather may result from drift and past competition.

The type of data and feasible experiments in ecology and cancer biology differ. With cancer patients, interventions and measurements must be carefully designed to minimize potential harm, while in ecological systems we are often constrained by feasibility, environmental protection, and funding. Laboratory and greenhouse systems sacrifice realism for control and may simulate pulsed resource environments that select for "cream-skimmer" phenotypes (Huntly et al.). Prior hypotheses can lead to publication bias of finding mainly what we are looking for (Wu). Ultimately, cancer biology rigor and ecological rigor are different but can and should be combined in cancer ecology (Plutynski). Both fields bring qualities that the other lacks: cancer biology is focused on molecular and cellular mechanisms underlying observed phenotypes, while ecologists unravel causal networks in complex systems that have been shaped by their evolutionary history.

Finally, as in all science, we must think about the goals and appropriate scale of inquiry of any biological investigation, whether the ultimate aim is fundamental understanding, forecasting, or treatment of disease. If we observe ecological phenomena like facilitation and competition (Noble et al.) or predation (Peplinski et al.) in cancer, do we need to understand the molecular details to effectively harness these phenomena for developing new treatment strategies? As genetic and molecular methods continue to unify the sciences, we think that the cross-talk between the methods, questions, and approaches between cancer ecology and traditional ecology will continue to increase to the benefit of both fields.

## AUTHOR CONTRIBUTIONS

FA outlined and drafted the initial version and edited later drafts. SA, EB, and CW contributed to the writing and editing. All authors contributed to the article and approved the submitted version.

## FUNDING

SA is supported by US Department of Defense CDMRP/PCRP (W81XWH-20-10353), the Patrick C. Walsh Prostate Cancer Research Fund, and the Prostate Cancer Foundation. CW was supported by NIH/NCI PSOC U54CA193489, NIH/NCI P30CA076292, and NIH/NCI U54 Supplement. FA was supported by U54 CA209978 NCA NIH and NIH U01 CA264620.

## ACKNOWLEDGMENTS

We thank the authors for their inspiring contributions to this Research Topic.

## REFERENCES

Amend, S. R., and Pienta K. J. (2015). Ecology meets cancer biology: the cancer swamp promotes the lethal cancer phenotype. *Oncotarget* 6, 9669–9678. doi: 10.18632/oncotarget.3430

Bissell, M. J., and Hines W. C. (2011). Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. *Nat. Med.* 17, 320–329. doi: 10.1038/nm.2328

Cairns, J. (1975). Mutation selection and the natural history of cancer. *Nature* 255, 197–200. doi: 10.1038/255197a0

Maley, C. C., Aktipis, A., Graham, T. A., Sottoriva, A., Boddy, A. M., Janiszewska, M., et al. (2017). Classifying the evolutionary and ecological features of neoplasms. *Nat. Rev. Cancer* 17, 605–619. doi: 10.1038/nrc.2017.69

Myers, K. V., Pienta, K. J., and Amend, R. S. (2020). Cancer cells and M2 macrophages: cooperative invasive ecosystem engineers. *Cancer Control.* 27:1073274820911058. doi: 10.1177/1073274820911058

Nowell, P. C. (1976). The clonal evolution of tumor cell populations. *Science* 194, 23–28. doi: 10.1126/science.959840

Pienta, K. J., McGregor, N., Axelrod, R., and Axelrod, E. D. (2008). Ecological therapy for cancer: defining tumors using an ecosystem paradigm suggests new opportunities for novel cancer treatments. *Transl. Oncol.* 1, 158–164. doi: 10.1593/tlo.08178

Whelan, C. J., and Gatenby, R. A. (2020). Special collection on ecological and evolutionary approaches to cancer control: cancer finds a conceptual home. *Cancer Control* 27:1073274820942356. doi: 10.1177/1073274820942356

**Conflict of Interest:** SA holds equity interest in Keystone Biopharma, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Ecology and Evolution

REVIEW
published: 28 April 2021
doi: 10.3389/fevo.2021.661583

# The Hallmarks of Cancer as Ecologically Driven Phenotypes

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Ecological fitness is the ability of individuals in a population to survive and reproduce. Individuals with increased fitness are better equipped to withstand the selective pressures of their environments. This paradigm pertains to all organismal life as we know it; however, it is also becoming increasingly clear that within multicellular organisms exist highly complex, competitive, and cooperative populations of cells under many of the same ecological and evolutionary constraints as populations of individuals in nature. In this review I discuss the parallels between populations of cancer cells and populations of individuals in the wild, highlighting how individuals in either context are constrained by their environments to converge on a small number of critical phenotypes to ensure survival and future reproductive success. I argue that the hallmarks of cancer can be distilled into key phenotypes necessary for cancer cell fitness: survival and reproduction. I posit that for therapeutic strategies to be maximally beneficial, they should seek to subvert these ecologically driven phenotypic responses.

**Keywords: ideal free distribution, metastasis, tumor microenvironment, fitness, niche construction theory**

OPEN ACCESS

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***Specialty section:***
*This article was submitted to Population and Evolutionary Dynamics, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *31 January 2021*
***Accepted:*** *24 March 2021*
***Published:*** *28 April 2021*

***Citation:***
*Somarelli JA (2021) The Hallmarks of Cancer as Ecologically Driven Phenotypes. Front. Ecol. Evol. 9:661583. doi: 10.3389/fevo.2021.661583*

## THE HALLMARKS OF CANCER AS ECOLOGICAL FITNESS PARAMETERS

Cancer is a breakdown in multicellularity that is driven by genetic mutation, leading ultimately to unchecked growth (Aktipis et al., 2015). This unchecked growth of populations of monoclonally derived cells, coupled with continued genetic instability/mutation, epigenetic dysregulation, and stochastic variation in gene expression and post-transcriptional regulation, often creates a genotypically- and phenotypically-diverse population of cancer cells. In the context of solid tumors, this diverse population of cancer cells resides within a complex and dynamic ecosystem that is spatially distinct in its inhabitants, resources, and geography. Cancer cells must interact with this ecosystem to ensure their survival [reviewed in Somarelli et al. (2020)]. The phenotypic traits necessary for the continued presence of cancer in the body are known as the *cancer hallmarks*, which were eloquently described in two landmark papers by Hanahan and Weinberg (Hanahan and Weinberg, 2011; **Figure 1**).

Interestingly, while these hallmark phenotypes are observed across all cancers, the underlying genetic/epigenetic mechanisms that drive these phenotypes are remarkably heterogeneous. Indeed, efforts by The Cancer Genome Atlas[1] and other consortia[2,3,4] to genomically characterize multiple cancer types have illuminated this tremendous genetic

[1]https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga
[2]https://platform.stjude.cloud/
[3]https://www.rarecancergenome.org/
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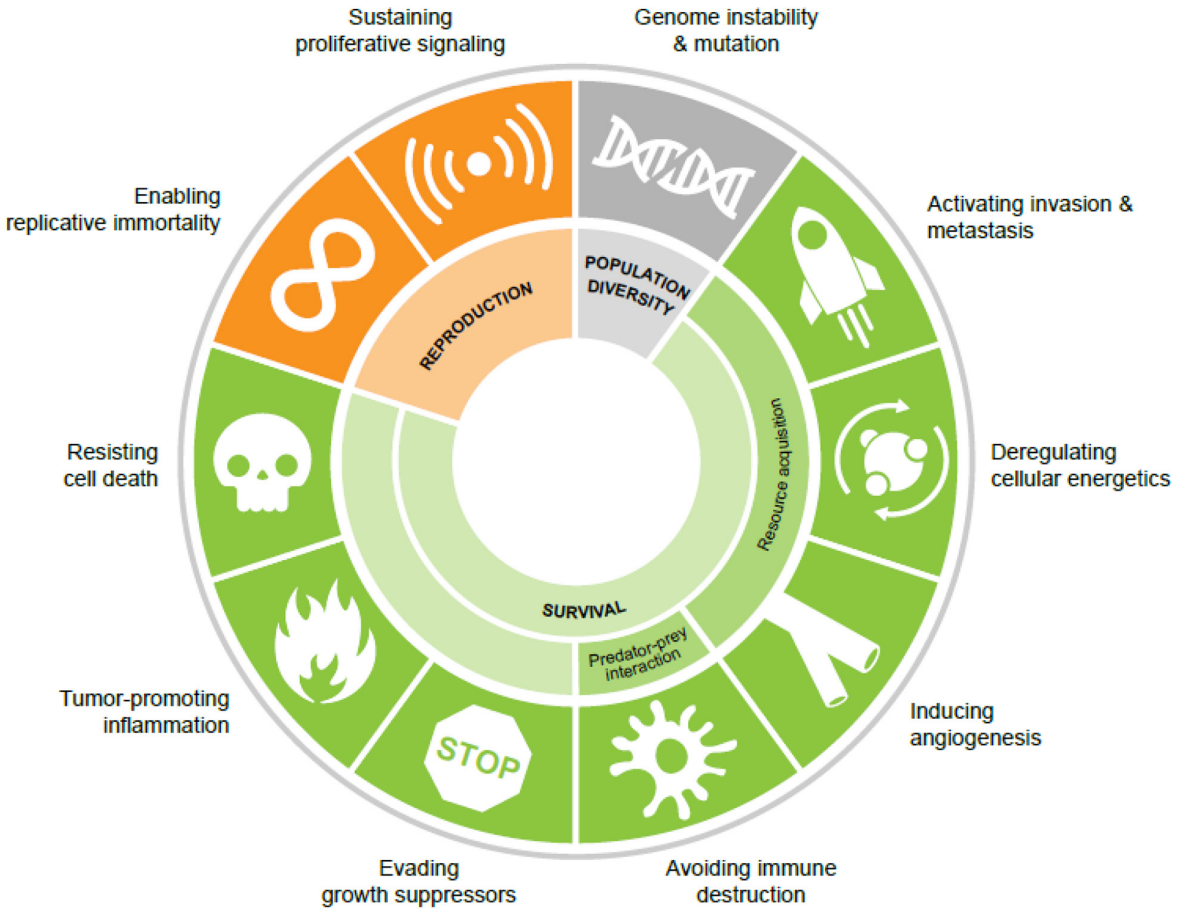


**FIGURE 1 |** The cancer hallmarks as ecological fitness parameters. Population diversity is driven by genome instability and mutation. Cancer cell fitness is governed by a series of survival phenotypes and the ability to reproduce (proliferation and replicative immortality).

and non-genetic diversity. The convergence of genotypically diverse individuals on a few key phenotypic traits is observed in ecological systems in the convergent evolution of phenotypes from genetically distinct species (Gatenby et al., 2011; Fortunato et al., 2017). Classic examples of this convergent evolution include the evolution of flight in insects, birds, and mammals (Chin and Lentink, 2016), the loss of sight and pigment in cave-dwelling fishes (Protas et al., 2006; Niven, 2008), and the evolution of fins and flippers in fishes and tetrapods (Fish and Lauder, 2017). Like these examples in nature, cancer cells, too, are constrained by their environments to converge on distinct phenotypic features that ensure their fitness within the ecology of the body. In this way, the cancer hallmarks represent the ecological fitness parameters of pro-survival and pro-reproduction (proliferation) phenotypes (**Figure 1**).

## CANCER CELLS EXIST WITHIN AN ECOLOGICAL SYSTEM

At its essence, what underlies the cancer hallmarks is an evolutionary fitness paradigm that describes key phenotypes necessary for survival and reproduction. In natural systems, the continued success of a species is defined by the fitness of its individuals. Fitness is the ability of an individual to survive and reproduce. At the population level, genetic and non-genetic variation within populations improves population-level fitness by increasing the likelihood that some individuals will survive and reproduce within a given ecological niche (Takahashi et al., 2018). An ecological niche includes all of the environmental conditions with which the individual interacts as well as the role played by the individual to shape its environment (Fath, 2018). Environmental conditions with which individuals interact include both biotic (e.g., predator/prey) and abiotic (e.g., geographic features) factors. These interactions dictate whether an individual maintains its fitness. Put simply, fitness is dependent upon a core set of phenotypes necessary for survival and successful reproduction. These core phenotypes can be achieved in myriad ways. For example, resource acquisition can be accomplished by altering food intake, migrating to new habitats/niches, or altering metabolism during periods of resource scarcity through hibernation or dormancy. Survival also includes diverse predatory escape/avoidance tactics.

Just as individual fitness is governed by interactions between individuals and their environments, cancers are also dependent upon the same core fitness phenotypes. With few exceptions (Andreoiu and Cheng, 2010), the vast majority of cancers originate from mutations within a single cell. Continued mutation of this clone during subsequent cell divisions leads to genetic diversity within a growing population of cancer cells. This genetic diversity is acted upon by selection for individual cells that can survive a specific ecological niche. Within solid tumors, the environment is spatially and temporally varied in the same way any natural environment would be, with multiple species co-existing within a dynamic, spatially diverse landscape (**Figure 2**). Like the natural world, the tumor is not merely a homogeneous cluster of cancer cells. Rather, the tumor is a pseudo-organ, comprised of both cancer and non-cancer cells co-existing together (**Figure 2**). These non-cancer cells, such as fibroblasts and other stromal cells (Sahai et al., 2020), endothelial cells (Hida et al., 2018), nerve cells (Banh et al., 2020), and immune cells (Binnewies et al., 2018) – which are often dysfunctional – contribute substantially to the tumor ecology by altering the resources and spatial geography of the tumor. In addition to the cells themselves, the local geography of the tumor is determined by vasculature, extracellular matrix components, resource availability, and tissue boundaries (**Figure 2**). This complex tumor environment shapes the survival phenotypes of resource availability and predation as well as the reproduction phenotype (**Figure 2**).

## THE ECOLOGY OF THE TUMOR SELECTS FOR CELLS THAT CAN SUCCESSFULLY FORAGE, AVOID PREDATION, MIGRATE, AND REPRODUCE

Resources, such as pro-survival signals, oxygen, and glucose are non-uniformly distributed throughout the tumor environment by the geography of the landscape and its non-cancer inhabitants (Milosevic et al., 1999; Rijken et al., 2000; Heaster et al., 2019; Zaidi et al., 2019). Neovascularization signals create a new blood supply that provides cancer cells with the oxygen, glucose, and growth factors that the cancer cells need for survival and reproduction. In addition to spatial heterogeneity in vasculature, resource distribution is also governed by the presence of non-cancer cells, many of which secrete signals in the form of growth factors, signaling ligands, or deposition of extracellular matrix components. Resource depletion can induce migratory/invasive properties (Yang et al., 2008; Chen et al., 2011; De Saedeleer et al., 2014). This relationship between resource depletion and migration is akin to the ecological

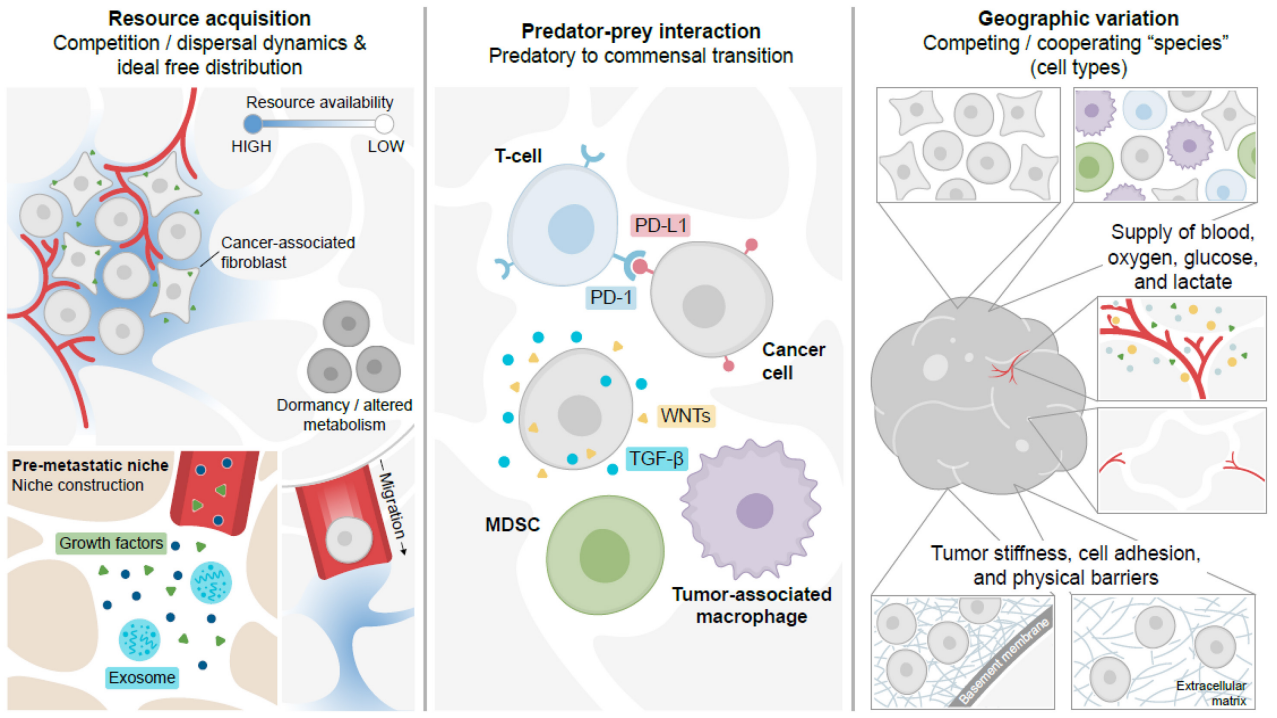


**FIGURE 2 |** Cancer cell survival within the dynamic ecology of the body is driven by resource acquisition, predator-prey interactions, and geography. Cancer cells within solid tumors maintain their survival through alterations in resource acquisition (cell-intrinsic and -extrinsic signals, dormancy, migration/dispersal) and niche construction. Cells must also avoid predation by the immune system through immune evasive and immune suppressive responses. These parameters are shaped by geography and resource distributions within the tumor or metastatic landscape. MDSC, myeloid-derived suppressor cell; PD-L1 is an immune evasive checkpoint molecule; WNTs and TGF-β are soluble immunomodulatory signals.

concept of the ideal free distribution in which individuals within a population redistribute in a given environment to equalize resource intake rates (Fretwell and Lucas, 1969). While the ideal free distribution concept is most often studied in the context of vertebrate animal behavior, this concept also applies across species – from invertebrates (Kelly and Thompson, 2000) to single-celled organisms (Moses et al., 2013) – in response to the resource distributions within ecosystems. A deeper understanding of how this ecological concept can be applied to solid tumor biology may help identify new treatments to inhibit metastasis by shifting tumor ecology toward an environment that inhibits pro-migratory phenotypes. For example, spatio-temporal knowledge of the resource limitations and carrying capacity of the tumor environment may improve timing of intermittent therapies to inhibit migration/invasion programs in response to resource depletion or maintain drug sensitivity. Consistent with this notion, monitoring of spatial tumor hypoxia is being applied to adaptive radiation therapy strategies (Gerard et al., 2019), and monitoring the timing of metabolic reprogramming during therapy has been used to define targeted vulnerabilities to prolong treatment response in preclinical models of breast cancer (Goldman et al., 2019).

Ecological systems are shaped not only by resource distribution, but also by the predatory-prey interactions within the environment (Friman et al., 2008). Predator-prey relationships have profound consequences for evolutionary fitness. Predators can influence fitness of their prey by inducing physiological, morphological, or behavioral responses (Schmitz, 2017) and by inducing evolutionary selective forces on the prey population (Schmitz and Trussell, 2016). While cancer cells cannot exhibit behavioral changes *per se*, the profound influence of predators on population structure occurs not only in ecological contexts, but also in cancer. For example, cytotoxic T cells shift the ecological balance toward cancer cell prey that are able to thwart this predatory-like behavior of the immune system. Cancer cells escape immune predation through (1) increased expression of checkpoint molecules that enhance cancer cell tolerance (Pardoll, 2012) and (2) secretion of immunosuppressive factors that alter the phenotype of immune cells (Ben-Baruch, 2006). The factors produced by the cancer cells shift the relationship between cancer cell and immune cell from a predator-prey to a commensal interaction in which the cancer cell benefits from the newly established relationship by surviving.

The fitness parameter of reproduction in the context of cancer is proliferation by way of mitotic cell division. To divide, a cancer cell first needs to survive. However, while survival is a pre-requisite for this reproductive cell division, additional signals are also necessary to ensure reproductive success; as in nature, survival alone does not guarantee reproduction (i.e., cell division). Indeed, disseminated cancer cells have been shown to remain undetectable for decades (Recasens and Munoz, 2019; Shen et al., 2020). The reasons for the lack of clinical detection are numerous, including a technical limit on detection (Hori and Gambhir, 2011), activation of cellular pathways related to dormancy and hibernation (Klein, 2020), immune surveillance (Swann and Smyth, 2007), and growth constraint due to limited resources [reviewed in Klein (2011)]. In some cases, however, a subset of disseminated cancer cells can reawaken their proliferative capacity and cause a clinically detectable relapse. This reawakening can be promoted by a change in environment. For instance, resource depletion within the tumor, such as hypoxia, lactate production, reactive oxygen species, or the presence of inflammatory cytokines can lead to p38/MAPK-mediated stress signaling (Kyriakis and Avruch, 1996). The p38/MAPK pathway is intimately connected to cell cycle arrest (Takenaka et al., 1998). Interestingly, p38 activation also promotes cellular migration (Hamanoue et al., 2016), which may enable dormant cancer cells to escape resource depletion in the primary tumor for the more resource-rich environment of the metastatic niche. This trade-off between proliferation and migration is analogous to the competition/dispersal trade-off observed in ecological contexts in which habitat stability (Pellissier, 2015), population density (Matthysen, 2005), and carrying capacity (Laroche et al., 2016) affect dispersal dynamics, with higher density, lower resource availability, and lower carrying capacity promoting dispersal. Integrating these parameters of tumor ecology into models of cancer metastasis may improve our understanding of the (1) timing of metastasis and (2) clonal heterogeneity expected in a given patient. Advances in genomic profiling of liquid biopsies (Gupta et al., 2017, 2020; Armstrong et al., 2019; Ignatiadis et al., 2021) provide a powerful system to monitor competition/dispersal tradeoffs longitudinally and adjust treatment to minimize dispersal. This competition/dispersal theory has also illustrated how genetically- and phenotypically-similar species can co-exist within an ecological niche (Yawata et al., 2014). Applying these models to cancer may provide insight into the cancer cell phenotypes that may be most likely to co-exist within tumors and could help identify rational treatment combinations.

The switch from stress signaling in the primary tumor to a more favorable environment in a metastatic site may induce reawakening of proliferative signals through a shift in the ratio of activated, phosphorylated (phospho) ERK:phospho-p38 signaling (Aguirre-Ghiso et al., 2003). For example, reduction in TGF-β signaling (Bragado et al., 2013) and urokinase plasminogen activator signaling (Aguirre-Ghiso et al., 2003) in metastatic sites leads to a decrease in phospho-p38 levels and increase in phospho-ERK. Remarkably, the balance between phospho-ERK-mediated proliferation/reproduction and phospho-p38-mediated cell cycle arrest/dormancy in cancer cells is also observed in hibernating animals. Cardiac muscle from hibernating thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) exhibits a significant upregulation in phospho-p38 during torpor and a low phospho-ERK: phospho-p38 ratio (Childers et al., 2019). Likewise, skeletal muscle samples from hibernating bats display a significant increase in phospho-p38 (Eddy and Storey, 2007). Hibernation is an adaptation that trades immediate reproduction under resource scarcity for a later chance at reproduction in times of greater resource availability (Willis, 2017). In the same way, cancer cell dormancy is a fitness tradeoff that limits immediate reproduction to ensure survival in response to resource depletion.

## CANCER CELLS AND NICHE CONSTRUCTION

An ecological niche is the interaction between an organism and its environment. While this interaction is most often discussed from the perspective of the influence of the environment on the organism, the concept of a niche also includes the influence of the organism on its environment. The ability of the organism to re-shape its environment to create a more optimal niche is referred to as niche construction (Laland et al., 2016). This concept of niche construction is defined by the following properties: (1) the organism significantly modifies its environment, and (2) these environmental modifications impact the selection pressures of the organism (Odling-Smee et al., 2013). For example, dam building by beavers dramatically alters the landscape, creating ponds and lakes where streams once were. This alteration in the landscape not only creates new habitat for the beavers and other species, but it also provides a selective force on beaver traits, such as their social behaviors and disease vulnerabilities (Naiman et al., 1988). Notably, this selective force outlasts the beavers who built the dam, providing a selective advantage beyond the current generation.

Like beavers, cancer cells also substantially modify their environments in my, and in doing so influence their selection. For instance, tumor cells alter the geography of their environments through deposition and proteolytic cleavage and clearance of extracellular matrix components (Winkler et al., 2020). These proteolytic enzymes, such as matrix metalloprotease 2 and 9, are prognostic for poorer clinical outcomes in several cancer types (Grignon et al., 1996; Sier et al., 1996; Li et al., 2017; Huang, 2018). Mechanistically, these proteases alter matrix stiffness (Das et al., 2017), facilitate migration by creating space (Krause and Wolf, 2015), and increase pro-survival signaling (Augoff et al., 2020). In addition to remodeling their geography, cancer cells also remodel their nutrient sources. For example, in this issue, Wu et al. describe the process whereby tumors accumulate high concentrations of proliferation-limiting resources. Likewise, secretion of pro-angiogenic factors, such as vascular endothelial growth factor, fibroblast growth factors, epidermal growth factor, and platelet-derived growth factor, mediates formation of new vasculature (Bergers and Benjamin, 2003). In addition, tumor cells exert pressure on other cell types within the habitat of the tumor. Release of soluble factors by cancer cells can induce fibroblasts to switch from tumor suppressing to tumor permissive [reviewed in Alkasalias et al. (2018)]. Cancer cells can also signal to the immune predators in the tumor through expression of immune checkpoints on the cancer cells, such as PD-L1 and CTLA4 (Pardoll, 2012), or through secretion of soluble immuno-suppressive factors, such as TGF-β (Wojtowicz-Praga, 2003), IL-10 (Kim et al., 2006), and soluble WNTs (Liang et al., 2014; Sun et al., 2017). This communication between cancer cells and immune subsets can lead to a restructuring of the immune landscape within the tumor toward a more tumor-tolerant environment.

Niche construction is not restricted to the local environment of the tumor. Systemic dissemination of signals also primes the pre-metastatic niche toward a cancer cell-permissive environment (Peinado et al., 2017; Doglioni et al., 2019). Signaling factors secreted by cancer cells can remodel distant sites for successful metastasis (Psaila and Lyden, 2009). The production of these secreted factors is also influenced by the local environment, thereby connecting local resource depletion and cell-to-cell crosstalk with distant niche construction in pre-metastatic organs. In mouse models of breast cancer, for example, tumor hypoxia led to the expression of lysyl oxidase, which induced recruitment of CD11b+ myeloid cells to remodel the collagen matrix of pre-metastatic lungs (Erler et al., 2009). Similarly, in preclinical models of lung adenocarcinoma and melanoma metastasis, conditioned media from tumor cells increased secretion of fibronectin in the pre-metastatic niche, which facilitated recruitment of tumor cell-promoting bone marrow-derived cells (Kaplan et al., 2005). Similar to secreted growth factors from tumor cells, exosomes carrying cargo throughout the body facilitate tumor cell seeding at pre-metastatic sites. These exosomes can harbor proteins (Costa-Silva et al., 2015; Hoshino et al., 2015), microRNAs (Rana et al., 2013; Zhou et al., 2014; Fong et al., 2015), and long non-coding RNAs, with impacts on the ecological niche, including extracellular matrix remodeling (Mu et al., 2013), angiogenesis and vascular permeability (Grange et al., 2011; Zeng et al., 2018), and immune cell populations (Liu et al., 2016; Wen et al., 2016). Unguided by an ecological perspective, many of the therapies that target these niche construction mechanisms have not been as successful as intended, and there is a remaining need to define the responses induced by cancer cells to remodel their niches, both locally and distally, at an individual patient level.

The most effective way to prevent the building of a dam is to remove the beavers before they cut down any trees. In the same way, early detection of cancer has been one of the most effective ways to improve cancer survival (Loud and Murphy, 2017). Despite their limitations, screening programs, particularly for colorectal, breast, cervical, prostate, skin, and other cancers have dramatically improved outcomes for cancer patients (Shieh et al., 2016; Loud and Murphy, 2017). While it has not been formally proven exactly how early detection has such a benefit, it is attractive to speculate that early removal of cancer cells prevents their (1) continued evolution toward more aggressive phenotypic states and (2) continued niche construction to create a permissive ecological landscape.

## THERAPY ALTERS THE CANCER CELL ECOLOGICAL NICHE, INDUCING RESPONSES THAT ARE BOTH BENEFICIAL AND DETRIMENTAL TO PATIENTS

Therapy substantially modifies the cancer cell population heterogeneity, fitness landscape, and ecology of the tumor

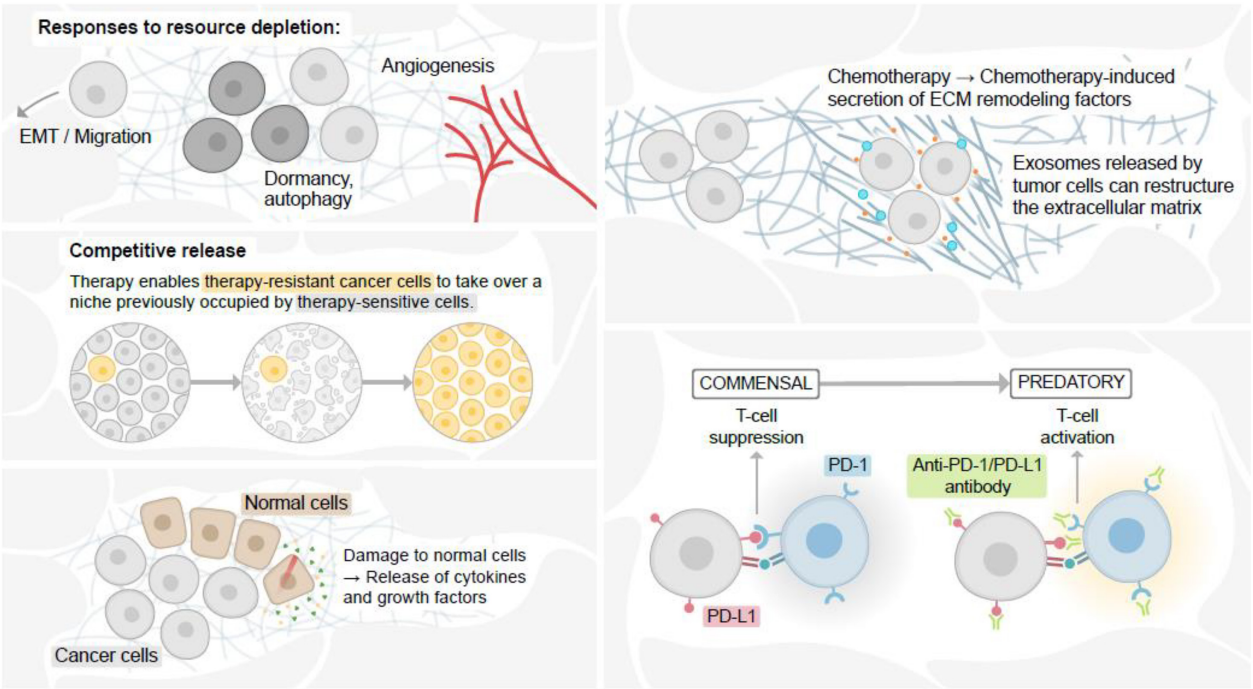


**FIGURE 3 |** Therapy alters the cancer cell ecological niche. Cellular responses to resource depletion in the face of therapy can induce epithelial-mesenchymal transition (EMT) and migration, altered resource acquisition, and angiogenesis **(upper L)**. Therapy-induced cellular damage can promote continued growth and tissue remodeling, providing growth factors and tissue space for remaining cancer cells to reoccupy the altered niche **(bottom L)**. Removal of drug-sensitive cells from an ecological niche can allow drug-resistant cells to take over the new niche **(top R)**. Immune checkpoint inhibition induces in the immune system a switch from a commensal to predatory.

(**Figure 3**). Whether by selection of a subclone with pre-existing resistance or the acquisition of a resistance mechanism in response to treatment, therapy often [though not always (Ding et al., 2012; Bashashati et al., 2013)] induces a strong selective bottleneck that enriches for resistant phenotypes within cancer cells. This can have profound impacts on population structure, as has been demonstrated in numerous cancer types through genomic profiling of longitudinal samples (Johnson et al., 2014; Gupta et al., 2017, 2020; Somarelli et al., 2017; Armstrong et al., 2019; Caswell-Jin et al., 2019; Roper et al., 2020). In addition, therapy-induced enrichment of resistant phenotypes can also promote additional aggressive features of cancer, such as altered resource acquisition (Lue et al., 2017; Xu et al., 2019; Gremke et al., 2020), dormancy (Kurppa et al., 2020; Ware et al., 2020), migration/invasion phenotypes (Takeuchi et al., 2015; Ware et al., 2016; Shah et al., 2017; Jolly et al., 2019), and immune evasion (Baghdadi et al., 2016; Ware et al., 2020).

In addition, the reshaping of the cancer cell population structure by therapeutic challenge can also alter the fitness landscape of the cell population in which removal of a drug-sensitive population allows drug-resistant cells to repopulate a newly vacant ecological niche (West et al., 2018; **Figure 3**). This ecological concept, known as competitive release, can be explained mechanistically by differences in energy expenditure within drug-sensitive and drug-resistant populations. In the case of cytotoxic chemotherapy, resistant cells expend substantial energy in response to the drug [reviewed in Silva et al. (2012) and Kam et al. (2014)]. This energy expenditure renders resistant cells less fit than sensitive cells. When the drug is removed, sensitive cells are able to outcompete the resistant cells for space within the newly available ecological niche.

While the goal of systemic therapy is to target the cancer cells, the therapy can also have unintended consequences on non-malignant cells within the ecological system, some of which can promote further aggressive features of the cancer cells. For example, treatment-induced damage to cells within the tumor microenvironment has been shown to release secreted factors that enhance cancer cell survival (Sun et al., 2012; Li et al., 2021). Likewise, chemotherapy can also remodel the surrounding geography of the extracellular matrix, leading to increased cancer cell survival (Bandari et al., 2018; **Figure 3**). Chemotherapy can also alter the immune landscape by damaging hematopoietic stem cell niches (Gardner, 1999), leading to immune suppression (Wu and Waxman, 2018). Chemotherapy has also been shown to suppress immune function through secretion of immunosuppressive factors, such as IL34 (Baghdadi et al., 2016) and granulocyte macrophage colony-stimulating factor (Takeuchi et al., 2015). Therapy-induced cancer cell phenotypic plasticity also induces a host of immunomodulatory signaling pathways (Alumkal et al., 2020). Unlike the mostly unintended effects of chemotherapy on the

"species" of immune cells within the tumor and the body, however, immunotherapy is specifically designed to reprogram the interaction between cancer cells and the immune system from a commensal to a predatory relationship (**Figure 3**). Ongoing and future work aimed at modeling these interactions using ecological frameworks (Griffiths et al., 2020) could improve trial design, predictive and prognostic power, and identify new mechanisms or treatment strategies aimed at prolonging the lives of cancer patients.

## LEVERAGING ECOLOGICAL RESPONSES TO GUIDE NOVEL THERAPEUTIC STRATEGIES

Viewing cancer from an ecological perspective can impact treatment paradigms. For instance, adaptive therapy in which treatment doses and schedules are adjusted based on the differential fitness of resistant and sensitive populations in the context of a drug has significantly prolonged tumor control in preclinical models of breast and ovarian cancers (Enriquez-Navas et al., 2016), with ongoing clinical trials in prostate cancer (Zhang et al., 2017) and other cancers [discussed in Cunningham et al. (2020)]. While these strategies have the potential to provide novel concepts to control disease, it is also imperative that we have a clear understanding of the relative fitness differences across resistance mechanisms and in different contexts. Adaptive therapy regimens may need to take into consideration both the frequency and relative fitness of resistance genotype/phenotype relationships (Schaper and Louis, 2014). For example, the "arrival of the frequent" (Schaper and Louis, 2014) suggests that frequent, but less fit phenotypes can become fixed in a population while other, rare phenotypes exist with increased fitness.

While the predominant focus of adaptive therapy has been on differential fitness and competition within cancer cell populations, other benefits of adaptive therapy may exist that are related to the tumor microenvironment. Lower drug doses may prevent toxicity to immune predatory cells. In the context of dying tumor cells, reduced lymphopenia may improve systemic immune response to localized and disseminated cancer cells. Similarly, lower drug concentrations within the tumor microenvironment may reduce the induction of a migratory/invasive phenotype in response to drug-mediated resource depletion.

In addition to adaptive therapy, strategies to target the tumor microenvironment and alter tissue ecology could be leveraged for novel combinations to inhibit both cancer cell-intrinsic and cell-extrinsic survival signals (Jin and Jin, 2020), alter tissue structure/geography (Erler et al., 2009; Juarez et al., 2012), and enhance immune predation (Opzoomer et al., 2019). Another approach may be to capitalize on dormancy as a response to therapy-mediated resource-depletion. For example, by using sequential treatment paradigms to first force cells into persistence/dormancy-like phenotypes, and then target these persistent cells with a secondary agent (Cipponi et al., 2020; Shen et al., 2020), it may be possible to prolong survival for patients with therapy-resistant or micrometastatic disease.

## CONCLUSION

The range of possible genetic solutions available to cancer cells in order to ensure survival and proliferation is vast. These innumerable possible solutions are constrained by the ecology of the individual patient, as well as the fundamental needs for survival and proliferation under stress, resulting in a set of phenotypic hallmarks. The hallmarks, at their essence, represent the phenotypic *solutions* for maintaining fitness within the ecological niche of the body. Ecologically informed therapeutic strategies can take advantage of these phenotypic responses required for fitness by using novel treatment approaches. To do this, the landscape of fitness parameters for each patient should be defined in order to identify rationale combinations or targets that control multiple aspects of cancer cell fitness. Beyond genetic drivers alone, therapeutic strategies should also consider the following in defining the fitness landscape of each patient: (1) identifying key resources, (2) defining the reproduction vs. dormancy/survival axis for tumors, (3) characterizing population heterogeneity, (4) quantifying dispersal likelihood, and (5) defining the predator/commensal state of the immune system. Broader partnership between ecologists and cancer researchers/physicians will help inform these strategies and could lead to further breakthroughs and innovation that capitalize on advances in spatially resolved genomics, measurements of the temporal dynamics of cancer cell populations, and an emerging arsenal of therapies that target both cancer cells and their habitats. Coupling these emerging technologies with ecologically informed models of cancer may enhance our ability to treat cancer as a chronic, but controllable illness that will substantially prolong the lives of cancer patients.

## AUTHOR CONTRIBUTIONS

JS conceptualized and wrote the manuscript.

## FUNDING

JS receives funding support from the Triangle Center for Evolutionary Medicine (TriCEM), the Department of Defense (W81XWH-18-1-0189), and the National Cancer Institute (5R01CA233585-02).

## ACKNOWLEDGMENTS

I wish to thank Ms. Ella Gunady for providing figure illustrations.

## REFERENCES


Aguirre-Ghiso, J. A., Estrada, Y., Liu, D., and Ossowski, L. (2003). ERK (MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK). *Cancer Res.* 63, 1684–1695.

Aktipis, C. A., Boddy, A. M., Jansen, G., Hibner, U., Hochberg, M. E., Maley, C. C., et al. (2015). Cancer across the tree of life: cooperation and cheating in multicellularity. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370:20140219. doi: 10.1098/rstb.2014.0219

Alkasalias, T., Moyano-Galceran, L., Arsenian-Henriksson, M., and Lehti, K. (2018). Fibroblasts in the tumor microenvironment: shield or spear? *Int. J. Mol. Sci.* 19:1532. doi: 10.3390/ijms19051532

Alumkal, J. J., Sun, D., Lu, E., Beer, T. M., Thomas, G. V., Latour, E., et al. (2020). Transcriptional profiling identifies an androgen receptor activity-low, stemness program associated with enzalutamide resistance. *Proc. Natl. Acad. Sci. U.S.A.* 117, 12315–12323. doi: 10.1073/pnas.1922207117

Andreoiu, M., and Cheng, L. (2010). Multifocal prostate cancer: biologic, prognostic, and therapeutic implications. *Hum. Pathol.* 41, 781–793. doi: 10.1016/j.humpath.2010.02.011

Armstrong, A. J., Halabi, S., Luo, J., Nanus, D. M., Giannakakou, P., Szmulewitz, R. Z., et al. (2019). Prospective multicenter validation of androgen receptor splice variant 7 and hormone therapy resistance in high-risk castration-resistant prostate cancer: the PROPHECY Study. *J. Clin. Oncol.* 37, 1120–1129. doi: 10.1200/JCO.18.01731

Augoff, K., Hryniewicz-Jankowska, A., and Tabola, R. (2020). Invadopodia: clearing the way for cancer cell invasion. *Ann. Transl. Med.* 8:902. doi: 10.21037/atm.2020.02.157

Baghdadi, M., Wada, H., Nakanishi, S., Abe, H., Han, N., Putra, W. E., et al. (2016). Chemotherapy-Induced il34 enhances immunosuppression by tumor-associated macrophages and mediates survival of chemoresistant lung cancer cells. *Cancer Res.* 76, 6030–6042. doi: 10.1158/0008-5472.CAN-16-1170

Bandari, S. K., Purushothaman, A., Ramani, V. C., Brinkley, G. J., Chandrashekar, D. S., Varambally, S., et al. (2018). Chemotherapy induces secretion of exosomes loaded with heparanase that degrades extracellular matrix and impacts tumor and host cell behavior. *Matrix Biol.* 65, 104–118. doi: 10.1016/j.matbio.2017.09.001

Banh, R. S., Biancur, D. E., Yamamoto, K., Sohn, A. S. W., Walters, B., Kuljanin, M., et al. (2020). Neurons release serine to support mRNA translation in pancreatic cancer. *Cell* 183, 1202.e25–1218.e25. doi: 10.1016/j.cell.2020.10.016

Bashashati, A., Ha, G., Tone, A., Ding, J., Prentice, L. M., Roth, A., et al. (2013). Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. *J. Pathol.* 231, 21–34. doi: 10.1002/path.4230

Ben-Baruch, A. (2006). Inflammation-associated immune suppression in cancer: the roles played by cytokines, chemokines and additional mediators. *Semin. Cancer Biol.* 16, 38–52. doi: 10.1016/j.semcancer.2005.07.006

Bergers, G., and Benjamin, L. E. (2003). Tumorigenesis and the angiogenic switch. *Nat. Rev. Cancer* 3, 401–410. doi: 10.1038/nrc1093

Binnewies, M., Roberts, E. W., Kersten, K., Chan, V., Fearon, D. F., Merad, M., et al. (2018). Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat. Med.* 24, 541–550. doi: 10.1038/s41591-018-0014-x

Bragado, P., Estrada, Y., Parikh, F., Krause, S., Capobianco, C., Farina, H. G., et al. (2013). TGF-beta2 dictates disseminated tumour cell fate in target organs through TGF-beta-RIII and p38alpha/beta signalling. *Nat. Cell Biol.* 15, 1351–1361. doi: 10.1038/ncb2861

Caswell-Jin, J. L., McNamara, K., Reiter, J. G., Sun, R., Hu, Z., Ma, Z., et al. (2019). Clonal replacement and heterogeneity in breast tumors treated with neoadjuvant HER2-targeted therapy. *Nat. Commun.* 10:657. doi: 10.1038/s41467-019-08593-4

Chen, J., Sprouffske, K., Huang, Q., and Maley, C. C. (2011). Solving the puzzle of metastasis: the evolution of cell migration in neoplasms. *PLoS One* 6:e17933. doi: 10.1371/journal.pone.0017933

Childers, C. L., Tessier, S. N., and Storey, K. B. (2019). The heart of a hibernator: EGFR and MAPK signaling in cardiac muscle during the hibernation of thirteen-lined ground squirrels. *Ictidomys tridecemlineatus*. PeerJ. 7:e7587. doi: 10.7717/peerj.7587

Chin, D. D., and Lentink, D. (2016). Flapping wing aerodynamics: from insects to vertebrates. *J. Exp. Biol.* 219(Pt 7), 920–932. doi: 10.1242/jeb.042317

Cipponi, A., Goode, D. L., Bedo, J., McCabe, M. J., Pajic, M., Croucher, D. R., et al. (2020). MTOR signaling orchestrates stress-induced mutagenesis, facilitating adaptive evolution in cancer. *Science* 368, 1127–1131. doi: 10.1126/science.aau8768

Costa-Silva, B., Aiello, N. M., Ocean, A. J., Singh, S., Zhang, H., Thakur, B. K., et al. (2015). Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat. Cell Biol.* 17, 816–826. doi: 10.1038/ncb3169

Cunningham, J., Thuijsman, F., Peeters, R., Viossat, Y., Brown, J., Gatenby, R., et al. (2020). Optimal control to reach eco-evolutionary stability in metastatic castrate-resistant prostate cancer. *PLoS One* 15:e0243386. doi: 10.1371/journal.pone.0243386

Das, A., Monteiro, M., Barai, A., Kumar, S., and Sen, S. (2017). MMP proteolytic activity regulates cancer invasiveness by modulating integrins. *Sci. Rep.* 7:14219. doi: 10.1038/s41598-017-14340-w

De Saedeleer, C. J., Porporato, P. E., Copetti, T., Perez-Escuredo, J., Payen, V. L., Brisson, L., et al. (2014). Glucose deprivation increases monocarboxylate transporter 1 (MCT1) expression and MCT1-dependent tumor cell migration. *Oncogene* 33, 4060–4068. doi: 10.1038/onc.2013.454

Ding, L., Ley, T. J., Larson, D. E., Miller, C. A., Koboldt, D. C., Welch, J. S., et al. (2012). Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature* 481, 506–510. doi: 10.1038/nature10738

Doglioni, G., Parik, S., and Fendt, S.-M. (2019). Interactions in the (Pre)metastatic niche support metastasis formation. *Front. Oncol.* 9:219. doi: 10.3389/fonc.2019.00219

Eddy, S. F., and Storey, K. B. (2007). p38 MAPK regulation of transcription factor targets in muscle and heart of the hibernating bat, *Myotis lucifugus*. *Cell Biochem. Funct.* 25, 759–765. doi: 10.1002/cbf.1416

Enriquez-Navas, P. M., Kam, Y., Das, T., Hassan, S., Silva, A., Foroutan, P., et al. (2016). Exploiting evolutionary principles to prolong tumor control in preclinical models of breast cancer. *Sci. Transl. Med.* 8:327ra24. doi: 10.1126/scitranslmed.aad7842

Erler, J. T., Bennewith, K. L., Cox, T. R., Lang, G., Bird, D., Koong, A., et al. (2009). Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. *Cancer Cell* 15, 35–44. doi: 10.1016/j.ccr.2008.11.012

Fath, B. (2018). *Encyclopedia of Ecology*, 2nd Edn. Amsterdam: Elsevier.

Fish, F. E., and Lauder, G. V. (2017). Control surfaces of aquatic vertebrates: active and passive design and function. *J. Exp. Biol.* 220(Pt 23), 4351–4363. doi: 10.1242/jeb.149617

Fong, M. Y., Zhou, W., Liu, L., Alontaga, A. Y., Chandra, M., Ashby, J., et al. (2015). Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. *Nat. Cell Biol.* 17, 183–194. doi: 10.1038/ncb3094

Fortunato, A., Boddy, A., Mallo, D., Aktipis, A., Maley, C. C., and Pepper, J. W. (2017). Natural selection in cancer biology: from molecular snowflakes to trait hallmarks. *Cold Spring Harb. Perspect. Med.* 7:a029652. doi: 10.1101/cshperspect.a029652

Fretwell, S. D., and Lucas, H. L. (1969). On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica* 19, 16–36. doi: 10.1007/BF01601953

Friman, V. P., Hiltunen, T., Laakso, J., and Kaitala, V. (2008). Availability of prey resources drives evolution of predator-prey interaction. *Proc. Biol. Sci.* 275, 1625–1633. doi: 10.1098/rspb.2008.0174

Gardner, R. V. (1999). Long term hematopoietic damage after chemotherapy and cytokine. *Front. Biosci.* 4:e47–e57.

Gatenby, R. A., Gillies, R. J., and Brown, J. S. (2011). Of cancer and cave fish. *Nat. Rev. Cancer* 11, 237–238. doi: 10.1038/nrc3036

Gerard, M., Corroyer-Dulmont, A., Lesueur, P., Collet, S., Cherel, M., Bourgeois, M., et al. (2019). Hypoxia imaging and adaptive radiotherapy: a state-of-the-art approach in the management of glioma. *Front. Med.* 6:117. doi: 10.3389/fmed.2019.00117

Goldman, A., Khiste, S., Freinkman, E., Dhawan, A., Majumder, B., Mondal, J., et al. (2019). Targeting tumor phenotypic plasticity and metabolic remodeling in adaptive cross-drug tolerance. *Sci. Signal.* 12:eaas8779. doi: 10.1126/scisignal.aas8779

Grange, C., Tapparo, M., Collino, F., Vitillo, L., Damasco, C., Deregibus, M. C., et al. (2011). Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. *Cancer Res.* 71, 5346–5356. doi: 10.1158/0008-5472.CAN-11-0241

Gremke, N., Polo, P., Dort, A., Schneikert, J., Elmshauser, S., Brehm, C., et al. (2020). mTOR-mediated cancer drug resistance suppresses autophagy and generates a druggable metabolic vulnerability. *Nat. Commun.* 11:4684. doi: 10.1038/s41467-020-18504-7

Griffiths, J. I., Wallet, P., Pflieger, L. T., Stenehjem, D., Liu, X., Cosgrove, P. A., et al. (2020). Circulating immune cell phenotype dynamics reflect the strength of tumor-immune cell interactions in patients during immunotherapy. *Proc. Natl. Acad. Sci. U.S.A.* 117, 16072–16082. doi: 10.1073/pnas.1918937117

Grignon, D. J., Sakr, W., Toth, M., Ravery, V., Angulo, J., Shamsa, F., et al. (1996). High levels of tissue inhibitor of metalloproteinase-2 (TIMP-2) expression are associated with poor outcome in invasive bladder cancer. *Cancer Res.* 56, 1654–1659.

Gupta, S., Hovelson, D. H., Kemeny, G., Halabi, S., Foo, W. C., Anand, M., et al. (2020). Discordant and heterogeneous clinically relevant genomic alterations in circulating tumor cells vs plasma DNA from men with metastatic castration resistant prostate cancer. *Genes Chromosomes Cancer* 59, 225–239. doi: 10.1002/gcc.22824

Gupta, S., Li, J., Kemeny, G., Bitting, R. L., Beaver, J., Somarelli, J. A., et al. (2017). Whole genomic copy number alterations in circulating tumor cells from men with abiraterone or enzalutamide-resistant metastatic castration-resistant prostate cancer. *Clin. Cancer Res.* 23, 1346–1357. doi: 10.1158/1078-0432.CCR-16-1211

Hamanoue, M., Morioka, K., Ohsawa, I., Ohsawa, K., Kobayashi, M., Tsuburaya, K., et al. (2016). Cell-permeable p38 MAP kinase promotes migration of adult neural stem/progenitor cells. *Sci. Rep.* 6:24279. doi: 10.1038/srep24279

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Heaster, T. M., Landman, B. A., and Skala, M. C. (2019). Quantitative spatial analysis of metabolic heterogeneity across in vivo and in vitro tumor models. *Front. Oncol.* 9:1144. doi: 10.3389/fonc.2019.01144

Hida, K., Maishi, N., Annan, D. A., and Hida, Y. (2018). Contribution of tumor endothelial cells in cancer progression. *Int. J. Mol. Sci.* 19:1272. doi: 10.3390/ijms19051272

Hori, S. S., and Gambhir, S. S. (2011). Mathematical model identifies blood biomarker-based early cancer detection strategies and limitations. *Sci. Transl. Med.* 3:109ra16. doi: 10.1126/scitranslmed.3003110

Hoshino, A., Costa-Silva, B., Shen, T. L., Rodrigues, G., Hashimoto, A., Tesic Mark, M., et al. (2015). Tumour exosome integrins determine organotropic metastasis. *Nature* 527, 329–335. doi: 10.1038/nature15756

Huang, H. (2018). Matrix Metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: recent advances. *Sensors* 18:3249. doi: 10.3390/s18103249

Ignatiadis, M., Sledge, G. W., and Jeffrey, S. S. (2021). Liquid biopsy enters the clinic - implementation issues and future challenges. *Nat. Rev. Clin. Oncol.* doi: 10.1038/s41571-020-00457-x [Epub ahead of print].

Jin, M. Z., and Jin, W. L. (2020). The updated landscape of tumor microenvironment and drug repurposing. *Signal. Transduct. Target Ther.* 5:166. doi: 10.1038/s41392-020-00280-x

Johnson, B. E., Mazor, T., Hong, C., Barnes, M., Aihara, K., McLean, C. Y., et al. (2014). Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science* 343, 189–193. doi: 10.1126/science.1239947

Jolly, M. K., Somarelli, J. A., Sheth, M., Biddle, A., Tripathi, S. C., Armstrong, A. J., et al. (2019). Hybrid epithelial/mesenchymal phenotypes promote metastasis and therapy resistance across carcinomas. *Pharmacol. Ther.* 194, 161–184. doi: 10.1016/j.pharmthera.2018.09.007

Juarez, P., Mohammad, K. S., Yin, J. J., Fournier, P. G., McKenna, R. C., Davis, H. W., et al. (2012). Halofuginone inhibits the establishment and progression of melanoma bone metastases. *Cancer Res.* 72, 6247–6256. doi: 10.1158/0008-5472.CAN-12-1444

Kam, Y., Das, T., Minton, S., and Gatenby, R. A. (2014). Evolutionary strategy for systemic therapy of metastatic breast cancer: balancing response with suppression of resistance. *Womens Health* 10, 423–430. doi: 10.2217/whe.14.23

Kaplan, R. N., Riba, R. D., Zacharoulis, S., Bramley, A. H., Vincent, L., Costa, C., et al. (2005). VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* 438, 820–827. doi: 10.1038/nature04186

Kelly, D. W., and Thompson, C. E. (2000). Epidemiology and optimal foraging: modelling the ideal free distribution of insect vectors. *Parasitology* 120(Pt 3), 319–327. doi: 10.1017/s0031182099005442

Kim, R., Emi, M., Tanabe, K., and Arihiro, K. (2006). Tumor-driven evolution of immunosuppressive networks during malignant progression. *Cancer Res.* 66, 5527–5536. doi: 10.1158/0008-5472.CAN-05-4128

Klein, C. A. (2011). Framework models of tumor dormancy from patient-derived observations. *Curr. Opin. Gene.t Dev.* 21, 42–49. doi: 10.1016/j.gde.2010.10.011

Klein, C. A. (2020). Cancer progression and the invisible phase of metastatic colonization. *Nat. Rev. Cancer* 20, 681–694. doi: 10.1038/s41568-020-00300-6

Krause, M., and Wolf, K. (2015). Cancer cell migration in 3D tissue: negotiating space by proteolysis and nuclear deformability. *Cell Adh. Migr.* 9, 357–366. doi: 10.1080/19336918.2015.1061173

Kurppa, K. J., Liu, Y., To, C., Zhang, T., Fan, M., Vajdi, A., et al. (2020). Treatment-induced tumor dormancy through YAP-Mediated transcriptional reprogramming of the apoptotic pathway. *Cancer Cell* 37, 104.e12–122.e12. doi: 10.1016/j.ccell.2019.12.006

Kyriakis, J. M., and Avruch, J. (1996). Sounding the alarm: protein kinase cascades activated by stress and inflammation. *J. Biol. Chem.* 271, 24313–24316. doi: 10.1074/jbc.271.40.24313

Laland, K., Matthews, B., and Feldman, M. W. (2016). An introduction to niche construction theory. *Evol. Ecol.* 30, 191–202. doi: 10.1007/s10682-016-9821-z

Laroche, F., Jarne, P., Perrot, T., and Massol, F. (2016). The evolution of the competition-dispersal trade-off affects alpha- and beta-diversity in a heterogeneous metacommunity. *Proc. Biol. Sci.* 283:20160548. doi: 10.1098/rspb.2016.0548

Li, H., Qiu, Z., Li, F., and Wang, C. (2017). The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. *Oncol. Lett.* 14, 5865–5870. doi: 10.3892/ol.2017.6924

Li, Y., Chen, Y., Miao, L., Wang, Y., Yu, M., Yan, X., et al. (2021). Stress-induced upregulation of TNFSF4 in cancer-associated fibroblast facilitates chemoresistance of lung adenocarcinoma through inhibiting apoptosis of tumor cells. *Cancer Lett.* 497, 212–220. doi: 10.1016/j.canlet.2020.10.032

Liang, X., Fu, C., Cui, W., Ober-Blobaum, J. L., Zahner, S. P., Shrikant, P. A., et al. (2014). beta-catenin mediates tumor-induced immunosuppression by inhibiting cross-priming of CD8(+) T cells. *J. Leukoc Biol.* 95, 179–190. doi: 10.1189/jlb.0613330

Liu, Y., Gu, Y., Han, Y., Zhang, Q., Jiang, Z., Zhang, X., et al. (2016). Tumor Exosomal RNAs promote lung pre-metastatic niche formation by activating alveolar epithelial TLR3 to Recruit neutrophils. *Cancer Cell* 30, 243–256. doi: 10.1016/j.ccell.2016.06.021

Loud, J. T., and Murphy, J. (2017). Cancer screening and early detection in the 21(st) century. *Semin. Oncol. Nurs.* 33, 121–128. doi: 10.1016/j.soncn.2017.02.002

Lue, H. W., Podolak, J., Kolahi, K., Cheng, L., Rao, S., Garg, D., et al. (2017). Metabolic reprogramming ensures cancer cell survival despite oncogenic signaling blockade. *Genes Dev.* 31, 2067–2084. doi: 10.1101/gad.305292.117

Matthysen, E. (2005). Density-dependent dispersal in birds and mammals. *Ecography* 28, 403–416. doi: 10.1111/j.0906-7590.2005.04073.x

Milosevic, M. F., Fyles, A. W., and Hill, R. P. (1999). The relationship between elevated interstitial fluid pressure and blood flow in tumors: a bioengineering analysis. *Int. J. Radiat. Oncol. Biol. Phys.* 43, 1111–1123. doi: 10.1016/s0360-3016(98)00512-4

Moses, M., Morris, D., and Qin, W. (2013). Greener on the other side of the fence: density-dependent habitat selection by a unicellular alga. *Evol. Ecol. Res.* 15, 809–828.

Mu, W., Rana, S., and Zoller, M. (2013). Host matrix modulation by tumor exosomes promotes motility and invasiveness. *Neoplasia* 15, 875–887. doi: 10.1593/neo.13786

Naiman, R. J., Johnston, C. A., and Kelley, J. C. (1988). Alteration of North American Streams by Beaver: the structure and dynamics of streams are changing as beaver recolonize their historic habitat. *BioScience* 38, 753–762. doi: 10.2307/1310784

Niven, J. E. (2008). Evolution: convergent eye losses in fishy circumstances. *Curr. Biol.* 18, R27–R29. doi: 10.1016/j.cub.2007.11.020

Odling-Smee, J., Erwin, D. H., Palkovacs, E. P., Feldman, M. W., and Laland, K. N. (2013). Niche construction theory: a practical guide for ecologists. *Q. Rev. Biol.* 88, 4–28. doi: 10.1086/669266

Opzoomer, J. W., Sosnowska, D., Anstee, J. E., Spicer, J. F., and Arnold, J. N. (2019). Cytotoxic chemotherapy as an immune stimulus: a molecular perspective on turning up the immunological heat on cancer. *Front. Immunol.* 10:1654. doi: 10.3389/fimmu.2019.01654

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12, 252–264. doi: 10.1038/nrc3239

Peinado, H., Zhang, H., Matei, I. R., Costa-Silva, B., Hoshino, A., Rodrigues, G., et al. (2017). Pre-metastatic niches: organ-specific homes for metastases. *Nat. Rev. Cancer* 17, 302–317. doi: 10.1038/nrc.2017.6

Pellissier, L. (2015). Stability and the competition-dispersal trade-off as drivers of speciation and biodiversity gradients. *Front. Ecol. Evol.* 3:52. doi: 10.3389/fevo.2015.00052

Protas, M. E., Hersey, C., Kochanek, D., Zhou, Y., Wilkens, H., Jeffery, W. R., et al. (2006). Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. *Nat. Genet.* 38, 107–111. doi: 10.1038/ng1700

Psaila, B., and Lyden, D. (2009). The metastatic niche: adapting the foreign soil. *Nat. Rev. Cancer* 9, 285–293. doi: 10.1038/nrc2621

Rana, S., Malinowska, K., and Zoller, M. (2013). Exosomal tumor microRNA modulates premetastatic organ cells. *Neoplasia* 15, 281–295. doi: 10.1593/neo.122010

Recasens, A., and Munoz, L. (2019). Targeting cancer cell dormancy. *Trends Pharmacol. Sci.* 40, 128–141. doi: 10.1016/j.tips.2018.12.004

Rijken, P. F., Bernsen, H. J., Peters, J. P., Hodgkiss, R. J., Raleigh, J. A., and van der Kogel, A. J. (2000). Spatial relationship between hypoxia and the (perfused) vascular network in a human glioma xenograft: a quantitative multi-parameter analysis. *Int. J. Radiat. Oncol. Biol. Phys.* 48, 571–582. doi: 10.1016/s0360-3016(00)00686-6

Roper, N., Brown, A. L., Wei, J. S., Pack, S., Trindade, C., Kim, C., et al. (2020). Clonal evolution and heterogeneity of osimertinib acquired resistance mechanisms in EGFR mutant lung cancer. *Cell Rep. Med.* 1:100007. doi: 10.1016/j.xcrm.2020.100007

Sahai, E., Astsaturov, I., Cukierman, E., DeNardo, D. G., Egeblad, M., Evans, R. M., et al. (2020). A framework for advancing our understanding of cancer-associated fibroblasts. *Nat. Rev. Cancer* 20, 174–186. doi: 10.1038/s41568-019-0238-1

Schaper, S., and Louis, A. A. (2014). The arrival of the frequent: how bias in genotype-phenotype maps can steer populations to local optima. *PLoS One* 9:e86635. doi: 10.1371/journal.pone.0086635

Schmitz, O. (2017). Predator and prey functional traits: understanding the adaptive machinery driving predator-prey interactions. *F1000Res.* 6:1767. doi: 10.12688/f1000research.11813.1

Schmitz, O. J., and Trussell, G. C. (2016). Multiple stressors, state-dependence and predation risk—foraging trade-offs: toward a modern concept of trait-mediated indirect effects in communities and ecosystems. *Curr. Opin. Behav. Sci.* 12, 6–11. doi: 10.1016/j.cobeha.2016.08.003

Shah, P. P., Dupre, T. V., Siskind, L. J., and Beverly, L. J. (2017). Common cytotoxic chemotherapeutics induce epithelial-mesenchymal transition (EMT) downstream of ER stress. *Oncotarget* 8, 22625–22639. doi: 10.18632/oncotarget.15150

Shen, S., Vagner, S., and Robert, C. (2020). Persistent cancer cells: the deadly survivors. *Cell* 183, 860–874. doi: 10.1016/j.cell.2020.10.027

Shieh, Y., Eklund, M., Sawaya, G. F., Black, W. C., Kramer, B. S., and Esserman, L. J. (2016). Population-based screening for cancer: hope and hype. *Nat. Rev. Clin. Oncol.* 13, 550–565. doi: 10.1038/nrclinonc.2016.50

Sier, C. F., Kubben, F. J., Ganesh, S., Heerding, M. M., Griffioen, G., Hanemaaijer, R., et al. (1996). Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. *Br. J. Cancer* 74, 413–417. doi: 10.1038/bjc.1996.374

Silva, A. S., Kam, Y., Khin, Z. P., Minton, S. E., Gillies, R. J., and Gatenby, R. A. (2012). Evolutionary approaches to prolong progression-free survival in breast cancer. *Cancer Res.* 72, 6362–6370. doi: 10.1158/0008-5472.CAN-12-2235

Somarelli, J. A., Gardner, H., Cannataro, V. L., Gunady, E. F., Boddy, A. M., Johnson, N. A., et al. (2020). Molecular biology and evolution of cancer: from discovery to action. *Mol. Biol. Evol.* 37, 320–326. doi: 10.1093/molbev/msz242

Somarelli, J. A., Ware, K. E., Kostadinov, R., Robinson, J. M., Amri, H., Abu-Asab, M., et al. (2017). PhyloOncology: understanding cancer through phylogenetic analysis. *Biochim. Biophys. Acta Rev. Cancer* 1867, 101–108. doi: 10.1016/j.bbcan.2016.10.006

Sun, X., Liu, S., Wang, D., Zhang, Y., Li, W., Guo, Y., et al. (2017). Colorectal cancer cells suppress CD4+ T cells immunity through canonical Wnt signaling. *Oncotarget* 8, 15168–15181. doi: 10.18632/oncotarget.14834

Sun, Y., Campisi, J., Higano, C., Beer, T. M., Porter, P., Coleman, I., et al. (2012). Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. *Nat. Med.* 18, 1359–1368. doi: 10.1038/nm.2890

Swann, J. B., and Smyth, M. J. (2007). Immune surveillance of tumors. *J. Clin. Invest.* 117, 1137–1146. doi: 10.1172/JCI31405

Takahashi, Y., Tanaka, R., Yamamoto, D., Noriyuki, S., and Kawata, M. (2018). Balanced genetic diversity improves population fitness. *Proc. Biol. Sci.* 285:20172045. doi: 10.1098/rspb.2017.2045

Takenaka, K., Moriguchi, T., and Nishida, E. (1998). Activation of the protein kinase p38 in the spindle assembly checkpoint and mitotic arrest. *Science* 280, 599–602. doi: 10.1126/science.280.5363.599

Takeuchi, S., Baghdadi, M., Tsuchikawa, T., Wada, H., Nakamura, T., Abe, H., et al. (2015). Chemotherapy-derived inflammatory responses accelerate the formation of immunosuppressive myeloid cells in the tissue microenvironment of human pancreatic cancer. *Cancer Res.* 75, 2629–2640. doi: 10.1158/0008-5472.CAN-14-2921

Ware, K. E., Gupta, S., Eng, J., Kemeny, G., Puviindran, B. J., Foo, W.-C., et al. (2020). Convergent evolution of p38/MAPK activation in hormone resistant prostate cancer mediates pro-survival, immune evasive, and metastatic phenotypes. *bioRxiv* [Preprint]. doi: 10.1101/2020.04.22.050385

Ware, K. E., Somarelli, J. A., Schaeffer, D., Li, J., Zhang, T., Park, S., et al. (2016). Snail promotes resistance to enzalutamide through regulation of androgen receptor activity in prostate cancer. *Oncotarget* 7, 50507–50521. doi: 10.18632/oncotarget.10476

Wen, S. W., Sceneay, J., Lima, L. G., Wong, C. S., Becker, M., Krumeich, S., et al. (2016). The biodistribution and immune suppressive effects of breast cancer-derived exosomes. *Cancer Res.* 76, 6816–6827. doi: 10.1158/0008-5472.CAN-16-0868

West, J., Ma, Y., and Newton, P. K. (2018). Capitalizing on competition: an evolutionary model of competitive release in metastatic castration resistant prostate cancer treatment. *J. Theor. Biol.* 455, 249–260. doi: 10.1016/j.jtbi.2018.07.028

Willis, C. K. R. (2017). Trade-offs influencing the physiological ecology of hibernation in temperate-zone bats. *Integr. Comp. Biol.* 57, 1214–1224. doi: 10.1093/icb/icx087

Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K. J., and Werb, Z. (2020). Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat. Commun.* 11:5120. doi: 10.1038/s41467-020-18794-x

Wojtowicz-Praga, S. (2003). Reversal of tumor-induced immunosuppression by TGF-beta inhibitors. *Invest. New Drugs* 21, 21–32. doi: 10.1023/a:1022951824806

Wu, J., and Waxman, D. J. (2018). Immunogenic chemotherapy: dose and schedule dependence and combination with immunotherapy. *Cancer Lett.* 419, 210–221. doi: 10.1016/j.canlet.2018.01.050

Xu, S., Ware, K. E., Ding, Y., Kim, S. Y., Sheth, M. U., Rao, S., et al. (2019). An integrative systems biology and experimental approach identifies convergence of epithelial plasticity, metabolism, and autophagy to promote chemoresistance. *J. Clin. Med.* 8:205. doi: 10.3390/jcm8020205

Yang, M. H., Wu, M. Z., Chiou, S. H., Chen, P. M., Chang, S. Y., Liu, C. J., et al. (2008). Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat. Cell Biol.* 10, 295–305. doi: 10.1038/ncb1691

Yawata, Y., Cordero, O. X., Menolascina, F., Hehemann, J. H., Polz, M. F., and Stocker, R. (2014). Competition-dispersal tradeoff ecologically differentiates recently speciated marine bacterioplankton populations. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5622–5627. doi: 10.1073/pnas.1318943111

Zaidi, M., Fu, F., Cojocari, D., McKee, T. D., and Wouters, B. G. (2019). Quantitative visualization of hypoxia and proliferation gradients within histological tissue sections. *Front. Bioeng. Biotechnol.* 7:397. doi: 10.3389/fbioe.2019.00397

Zeng, Z., Li, Y., Pan, Y., Lan, X., Song, F., Sun, J., et al. (2018). Cancer-derived exosomal miR-25-3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis. *Nat. Commun.* 9:5395. doi: 10.1038/s41467-018-07810-w

Zhang, J., Cunningham, J. J., Brown, J. S., and Gatenby, R. A. (2017). Integrating evolutionary dynamics into treatment of metastatic castrate-resistant prostate cancer. *Nat. Commun.* 8:1816. doi: 10.1038/s41467-017-01968-5

Zhou, W., Fong, M. Y., Min, Y., Somlo, G., Liu, L., Palomares, M. R., et al. (2014). Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. *Cancer Cell* 25, 501–515. doi: 10.1016/j.ccr.2014.03.007

**Conflict of Interest:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 05 May 2021
doi: 10.3389/fevo.2021.653622

# Oversupply of Limiting Cell Resources and the Evolution of Cancer Cells: A Review

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Cancer prevention is superior to cancer treatment—indeed, understanding and controlling cancer risk is a key question in the fields of applied ecology and evolutionary oncology. Ecological cancer risk models offer the dual benefit of being generalizable across cancer types, and unveiling common mechanisms underlying cancer development and spread. Understanding the biological mechanisms of cancer risk may also guide the design of interventions to prevent cancer. Ecological considerations are central to many of these mechanisms; as one example, the ecologically-based hypothesis of metabolic cancer suppression posits that restricted vascular supply of limiting resources to somatic tissues normally suppresses the evolution of somatic cells toward cancer. Here we present a critical review of published evidence relevant to this hypothesis, and we conclude that there is substantial evidence that cancer risk does increase with an abnormal excess of limiting cell resources, including both dietary macronutrients as well as certain micronutrients.

**Keywords: evolutionary oncology, cancer ecology, cancer prevention, resource oversupply, limiting resources**

OPEN ACCESS

***Edited by:***
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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *14 January 2021*
***Accepted:*** *08 April 2021*
***Published:*** *05 May 2021*

***Citation:***
*Wu DJ (2021) Oversupply of Limiting Cell Resources and the Evolution of Cancer Cells: A Review. Front. Ecol. Evol. 9:653622. doi: 10.3389/fevo.2021.653622*

## 1. INTRODUCTION

Cancer is the result of an evolutionary process. This is a process fueled by mutation and shaped by ecology (Reynolds et al., 2020), making the assessment and reduction of cancer risk an important problem in the fields of applied ecology and evolution. Consequently, a evolutionary approach to cancer modeling can improve models for individualized risk assessment, which are currently in need of improvement (Louro et al., 2019). Moreover, forming a theoretical account of the ecological mechanisms of cancer risk can guide the design of evolutionarily-enlightened interventions to prevent cancer.

The difference between indolent and aggressive cancers may sometimes lie not within the tumor itself, but rather in the tissue micro-environment where the tumor is growing. The ecology of the tissue microenvironment is central to understanding and intercepting cancer risk in two ways: Firstly, while mutations are stochastic and unpredictable, ecological effects on evolutionary trajectories are deterministic and predictable (Lenski, 2017; Barrick et al., 2020)—especially in cases of convergent evolution, such as the cellular evolution of cancer (Fortunato et al., 2017). Secondly, unlike mutation, tissue ecology is a modifiable source of cancer risk; understanding it can help us to not only predict the risk of cancer, but also take proactive steps to reduce it.

In this review, we focus on resource supply and limitations in the tumor microenvironment. Based on systems biology simulation models, it was proposed that in the somatic ecology of tissues, an abnormal excess of limiting energetic resources may increase cancer risk in the affected tissues (Wu et al., 2019). Indeed, a consortium of scientists identified responses to energetic resource

limitations as one of two primary ways to classify neoplasms (Maley et al., 2017). Here, we broaden the scope of this ecological idea to include not only energetic resources, but any resources that can be limiting for cell proliferation. We undertake a critical review of published empirical evidence relevant to testing this broader hypothesis.

## 2. MECHANISM

A foundational principle of ecology is Liebig's Law, which states that growth is controlled not by the total amount of all resources available, but by the scarcest (limiting) resource type (Egli and Zinn, 2003; Shapiro et al., 2018). For a given population, a resource type is limiting if an increase in its availability increases growth. Both in classical species ecology, and in somatic cell ecology, there is only one limiting resource type per population, but this is may vary over time, or between tissues. The single resource that is likely to be limiting for most somatic cells at most times is energy for biosynthesis, growth, and division. This is, however, not a universal rule; other limiting resources may include micronutrients, growth signals, oxygen, and many others. The relation between cancer development and resource oversupply is an extension of Leibig's law; cancer cells are treated as organisms whose growth is unlocked by resource surplus.

Heretofore, in considering cancer risk, much attention has been focused on the driver mutations in somatic cells that are believed to trigger oncogenesis. However, it has become clear that such mutations often do not drive oncogenesis, but instead remain safely "parked" in normal tissues (Tomasetti, 2019; Nam et al., 2020; Solary and Laplane, 2020). These observations support the notion that cancer prevention can be viewed as an attempt to change the selective pressures within tissues to prevent or delay cancer (Maley et al., 2011). It is still unknown what selective pressures might direct driver mutations toward malignancy; this is a fundamental open question in the science of cancer prevention. One potential explanation is the hypothesis of metabolic cancer suppression, which is based on known epidemiology of cancer risk. According to this hypothesis, restricted vascular supply of resources to somatic tissues normally limits resources critical to cell proliferation, and thereby suppresses cellular evolution toward cancer, even in the presence of driver mutations (**Figure 1**). Under this framework, the importance of driver mutations is attributable only to their impact on cellular fitness in the context of the tumoral microenvironment. This accords with an earlier mathematical model (Beerenwinkel et al., 2007), which suggested that the waiting time to cancer depends strongly on the selective advantage ($s$) conferred by oncogenic driver mutations, with the average waiting time proportional to $\frac{1}{s}$.

In computer simulations, cancer driver mutations quickly caused cancer in microenvironments that were oversupplied with limiting cell resources, but had little effect in tissues with normally restricted supplies of those resources (Wu et al., 2019). These ecological effects on cancer risk are consistent with our general understanding of how ecology shapes evolution. Several authors have noted that both in classical species ecology and in the tissue ecology of somatic cells, it is only populations in resource-rich environments that typically evolve the rapid-proliferation life histories that rely on rapid resource consumption (Alfarouk et al., 2013; Ducasse et al., 2015). Therefore, according to the hypothesis of metabolic cancer suppression, even cell resources that are healthy and essential in normal quantities can become carcinogenic in excess.

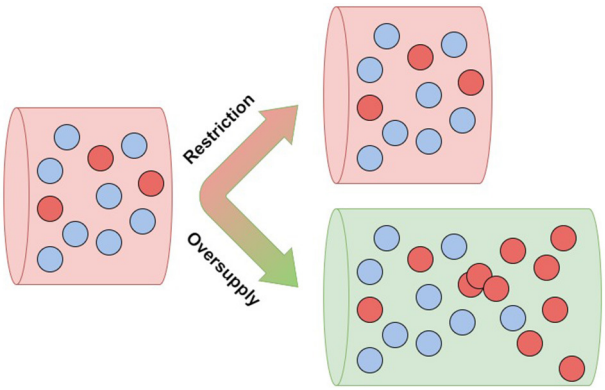


**FIGURE 1 |** Resource oversupply unlocks clonal expansion—aggressive clones (red) flourish in an abundant environment (green).

The eco-evolutionary effects of resource availability are difficult to observe in healthy tissue that is evolving toward cancer, but have been observed in cancer itself. Cancer cells that could gain a fitness advantage by exploiting a given resource typically evolve to do so only when that resource is available in their local microenvironment. For example, in breast cancer, elevated expression of estrogen receptor can increase cell fitness and proliferation, but only in the presence of adequate estrogen, which is supplied through vascular delivery. Cell phenotyping has shown that within a breast tumor, there is a strong correlation between cells' access to vascular delivery, and with their evolved expression of estrogen receptor (Lloyd et al., 2014). Such local effects on the evolution of cancer cells, as described above, suggest that even when a limiting resource is at normal levels in blood, vascular abnormalities creating excess local blood flow might increase cancer risk in the locally affected tissues. There are published observations suggesting that local vascular abnormalities increasing blood flow do, in fact, increase localized cancer risk in humans (Feinmesser et al., 1997; Lapidoth et al., 2006; Blatt et al., 2019). However, such local tissue effects are unlikely to be generally important to cancer prevention and control. In contrast, systemic excess of resources that are often limiting for cell proliferation affect more tissues and organs, and do so in many more people. Such systemic excess is likely to be important to cancer prevention and control, and it will be the focus of this review.

## 3. LIMITING RESOURCES

Although there are few empirical studies investigating the effect of *general* limiting resources on cancer development, there is

**TABLE 1 |** Some examples of evidence supporting the importance of limiting resources in cancer control.

| Limiting resource | Cancer type | References |
|---|---|---|
| Glucose | Prostate | Marrone et al. (2019) |
| | Gallbladder | Navarro et al. (2019) |
| | Colorectal | Yang et al. (2016) |
| | Pancreatic | Zhang et al. (2020) |
| Growth Factors | Prostate | Watts et al. (2019) |
| | Breast | Lloyd et al. (2014) |
| | Colorectal | Yamamoto et al. (2017) |
| | Ovarian | Brokaw et al. (2007) |
| Micronutrients | Prostate | Perez-Cornago et al. (2020) |
| | Skin | Whitnall et al. (2006) |
| | Bladder | Torti et al. (1998) |
| | Renal | Ba et al. (2011) |

a rich body of literature investigating the carcinogenic effect of *specific* resources which are likely to be limiting (**Table 1**). We consider these studies here, not to offer a comprehensive consideration of all of the possible limiting resources within the tumor microenvironment, but instead to illustrate the myriad ways in which resource limitations may arise and be subverted.

## 3.1. Glucose

The single class of resources that is likely to be limiting for most somatic cells at most times is energy; used for biosynthesis, growth, and division. This energy is supplied primarily by blood glucose, the supply of which is tightly regulated in normal physiology. In accordance with Liebig's law, researchers hypothesize that glucose oversupply may be associated with cancer risk.

In support of this hypothesis, it has been found that while hyperglycemia and diabetes were not significantly associated with total prostate cancer incidence, glycemia values above the normal range were associated with increased risk of lethal prostate cancer, and with prostate cancer mortality (Marrone et al., 2019). Similarly, a study of the aggressiveness of gallbladder cancer using circulating glucose-to-lymphocyte ratio (GLR) as an indicator of glucose availability, found that preoperative GLR was an independent predictor of survival (Navarro et al., 2019). A similar effect of blood glucose on cancer aggressiveness was reported for colorectal cancer (Yang et al., 2016), and also for pancreatic cancer (Zhang et al., 2020).

These consistent effects of glucose level on cancer risk are understandable through an eco-evolutionary perspective of carcinogenesis as the Darwinian evolution of somatic cells into cancer cells. Abnormally elevated glucose in the cell microenvironment alters the selective forces on cells in two ways: Firstly, excess glucose directly selects for cells with abnormally accelerated growth and proliferation that disproportionately benefit from this energetic windfall. Secondly, this hyperglycemic microenvironment allows neoplastic cells to gain a further fitness advantage over normal cells by generating energy through anaerobic glycolysis. While less efficient in glucose use than aerobic cellular respiration, this glycolytic pathway is an allelopathic strategy—it generates an acidic and toxic microenvironment that is more toxic to competing normal cells than to the cancer cells themselves (Gillies et al., 2008). In doing so, this destructive cell phenotype removes both competitive and physical barriers, thereby accelerating clonal expansion. These considerations, supported by empirical evidence and mathematical modeling, led some to conclude that elevated glucose consumption is a necessary cell phenotype for the formation of metastatic cancers (Gillies et al., 2008).

## 3.2. Inorganic Micronutrients

In addition to oxidizable energy substrates such as glucose, several inorganic trace nutrients, such as iron and phosphate, are also potentially limiting for cell proliferation, and are also over-consumed by rapidly dividing cells. According to Liebig's law, in those patients and tissues in which energy is the limiting resource, these trace nutrients are not limiting, thus their availability will not greatly affect cancer risk. However, in those patients with elevated blood glucose, cell proliferation is unlikely to be limited by energy availability, and so may be limited instead by crucial inorganic trace nutrients. In areas experiencing the current epidemic of obesity and metabolic syndrome, this situation may be common.

Iron is critical to cell proliferation because of its key roles in energy production and the biosynthesis of DNA and RNA. Like glucose, iron homeostasis is highly regulated, and its dysregulation has been associated with carcinogenesis (Weinberg, 1996; Torti and Torti, 2013). Both infections and neoplasms consist of rapidly dividing pathogenic cells, and host physiology defends against both infections and neoplasms by sequestering the iron required for rapid cell proliferation (Weinberg, 1984). Hosts respond to both infections and neoplasms by lowering their plasma iron levels; consequently, we expect that iron supplements enhance both microbial and neoplastic cell growth. Long-established animal experiments have demonstrated that experimental oversupply of iron by tissue injection can induce cancer at the injection site (Richmond, 1959). This is despite the fact the fact that physiological levels of iron are neither mutagenic nor carcinogenic (Weinberg, 1984). Substantial evidence from multiple studies suggests that abnormal iron excess is closely associated with tumorigenesis in multiple types of human cancer (Chen et al., 2019). In humans, excess tissue accumulation of iron due to food additives and iron supplements may be contributing to increased risk of cancer generally (Davoodi et al., 2016).

A second inorganic cell nutrient implicated in oncogenesis is phosphate. Evidence suggests that excess cellular phosphate, associated with dysregulated phosphate metabolism, acts as a growth-promoter in various human and experimental models of tumors (Brown and Razzaque, 2018). In a prospective study of multiple serum biomarkers potentially indicating risk of prostate cancer in a large cohort of men, phosphate was the only biomarker significantly positively correlated with prostate cancer during long term follow-up (Perez-Cornago et al., 2020). In an example of cancer niche construction, the phosphate proclivities of tumors are reflected in their tendency

to accumulate more phosphorus in their microenvironment compared to normal tissue. In mouse models of spontaneous cancer, *in vivo* measurements of several aspects of the chemical tumor microenvironment revealed that the greatest difference of tumor tissue compared to normal tissue was not the well-established chemical differences in oxygen and pH, but rather, differences in extracellular concentration of inorganic phosphate (Bobko et al., 2017). Levels of inorganic phosphate ($P_i$) were more than two-fold higher in tumor microenvironments vs. normal tissue. Moreover, $P_i$ concentration was the only parameter that allowed for discrimination between non-metastatic and highly metastatic tumors (Bobko et al., 2017).

### 3.3. Growth Factors

In addition to the nutrients discussed above, other candidates for resources limiting to cell proliferation include various endogenous signals. Similarly to nutrients, endogenous signaling molecules are received only through vascular delivery, and are often are taken up faster by hyperproliferating cells.

For example, insulin-like growth factors (IGFs) have been implicated in the etiology of several cancers. Epidemiological evidence implicates IGFs in risk for prostate, breast, colorectal cancer, and possibly thyroid cancer—IGF1 is consistently positively associated with an increased risk of these cancers (Watts et al., 2019). Estrogen is another endogenous signaling molecule that can be a limiting proliferation resource for some female reproductive cancers, in particular estrogen receptor positive (ER+) breast cancer. Within an ER+ tumor, estrogen dependence and uptake may vary from 1 to 100% of cells. It has been hypothesized that expression of estrogen receptors is positively selected only if estrogen is present in the microenvironment, and further, that tumor regions with higher blood flow would contain larger numbers of ER+ cells than areas of low blood flow (Lloyd et al., 2014). By examining the spatial distribution within tumors of ER+ and ER- cells relative to blood vessel area, the authors found a strong positive correlation between vascular area and ER expression. The authors concluded that ER expression, specifically by well-vascularized tumor cells, resulted from different selection pressures in well-vascularized regions. These results suggest that an abnormal excess of circulating estrogen could also lead to abnormal cell proliferation and to increased cancer risk.

That hypothesis has been supported by two unintended observational "experiments" on patterns of reproductive cycling in women. The first natural phenomenon involving abnormally increased estrogen exposure in women (relative to ancestral conditions) resulted from the introduction of agriculture and birth control. In the ancestral environment that humans are adapted to, women undergo relatively few ovulatory cycles with their accompanying surges of endogenous estrogen. In contrast, contemporary American women start cycling younger and cycle more often, resulting in about triple the lifetime ovulatory cycles as were typical of pre-agricultural women. Based on a theoretical model, this translates into a risk of breast cancer by age 60 that is about 100 times as high as that of pre-agricultural women, and this model prediction is consistent with the available epidemiological data (Eaton et al., 1994). The second phenomenon resulted from the introduction of hormone replacement therapy (HRT) to treat the symptoms of menopause. Multiple observational studies have showed an increased risk of breast cancer with multi-year use of HRT (Franceschini et al., 2020). These authors argue that this increased cancer risk has resulted not only from abnormally extended exposure to estrogen, but also from use of some older drugs with excessively high levels of estrogens. The combined weight of this evidence suggests that, as in the case of the nutrients discussed above, it may not be correct to consider estrogen to be carcinogenic *per se*, but rather to consider an abnormal excess of estrogen exposure to be carcinogenic.

## 4. DISCUSSION

In conclusion, a large body of evidence indicates that cancer risk does increase with an abnormal excess of limiting cell resources, including both exogenous dietary factors, and endogenous signaling molecules. Among dietary factors, this effect is most widely investigated in energetic macronutrients, which can upset the normal energy balance and create hyperglycemia, but the supporting evidence is also strong for at least two limiting micronutrients: iron and phosphate. The idea that normal and necessary nutrients could sometimes have a carcinogenic effect may seem paradoxical, but it should not be too surprising—when pathology is plotted as a function of various physiological measurements or dietary inputs, the result is often a U-shaped response curve, with pathology increasing as physiology becomes abnormally extreme in either direction.

Ideally, a systematic review of an association between resource oversupply and cancer outcomes should compare the number of studies that reported a positive association vs. those that found a negative or no association (Pati and Lorusso, 2018). While we have, to our best knowledge, included an unbiased sample of all relevant literature, few empirical studies have been designed specifically to test the hypothesis we examine. Instead, much of the available evidence is observational, or was incidental to the focus of the reported study. We found no published negative reports regarding association between dietary excess and cancer outcomes, but this could reflect either a limited literature, or a reporting bias toward positive association. We are optimistic that if the hypothesis addressed here gains plausibility, there will soon be more empirical research directly targeted at investigating resource oversupply.

### 4.1. Exogenous and Endogenous Resources

It is difficult to disentangle the role of endogenous signals from that of exogenous resources, because levels of the two are often highly correlated. Limiting resources are closely regulated physiologically, so that extreme levels of intake will cause a rapid increase in regulatory signaling molecules.

However, there is a fundamental difference between the constraints on carcinogenesis resulting from limited signaling molecules compared to that of limited exogenous nutritional resources—the latter has a more fundamental and robust causal

effect. Indeed, the evolution of independence from endogenous signals and factors that would normally limit proliferation is considered a canonical hallmark of cancer (Hanahan and Weinberg, 2000). Therefore, restricted levels of endogenous signals can only transiently impede the evolution of cancer. In contrast, conservation of energy dictates that cells cannot evolve away from dependence on energy and materials for growth and proliferation. Thus, restricted levels of exogenous limiting resources offer a more robust and important obstacle to somatic cell evolution toward cancer. As an example of this difference between the risks from exogenous resources vs. endogenous signals, normal somatic cells do not proliferate in the absence of insulin or of insulin-like growth factor 1 (IGF1). However, evolving cancer cells can evade this dependence, often through a single mutation. Tumors with this property have been described as resistant to caloric restriction, but in fact, animal experiments have only established their resistance to low serum levels of insulin and IGF1, and not to low levels of glucose itself (Kalaany and Sabatini, 2009). The problem of disentangling the effects of endogenous signals vs. exogenous resources is especially acute for glucose. Hyperglycemia, with its comorbidities metabolic syndrome and obesity, increases cancer risk not only through oversupply of glucose to tissues, but also through abnormal levels of IGF1, leptin, adiponectin, steroid hormones, insulin, and sirtuins (Hursting and Berger, 2010). As a mechanism of cancer risk, and as an opportunity for cancer prevention, serum glucose may prove to be the most important of these multiple intercorrelated risk factors.

## 4.2. Aggressive and Indolent Cancers

A key difficulty in cancer screening is the onerous diagnostic task of distinguishing between indolent cancers and clinically aggressive cases that demand immediate treatment (Srivastava et al., 2016). This problem is especially pressing for prostate cancer, which simultaneously proffers many cases of aggressive and dangerous disease, as well as many cases of over-diagnosed and over-treated tumors (Da Huang et al., 2020). Efforts at molecular characterization have not yet found mutations or other cell-intrinsic markers of cancer aggressiveness, and detailed consideration of tissue ecology may help. Indeed, it has been shown that the aggressiveness of prostate cancer is associated with circulating levels of several limiting cell resources, including glucose (Marrone et al., 2019), iron (Choi et al., 2008), and phosphate (Bobko et al., 2017). Given that resource oversupply may act as the "enabler" of carcinogenic mutations and clonal expansion, monitoring and analysis of the tumor microenvironment offers a promising method by which to identify clones which are likely to be aggressive.

## 4.3. Caloric Restriction and Dietary Regimens

Consistent with the hypothesis that excess blood glucose drives oncogenesis, a recent review concluded that the internal tissue environment determines whether cancers progress to advanced disease, and that glucose availability is an important component of this somatic ecology (Holly et al., 2019). Furthermore, a meta-analysis comparing people with and without diabetes found that diabetes was associated with substantial premature death from several types of cancer, independent of other major risk factors (Collaboration, 2011). These included cancers of the liver, pancreas, ovary, colorectum, lung, bladder, and breast. A prospective study also found blood glucose to predict risk of breast cancer (Muti et al., 2002). This work suggests that controlling blood glucose levels by managing caloric intake and increasing physical activity is likely to confer protective benefits for many types of cancer in economically developed areas (Giovannucci, 1999). Indeed, caloric restriction dietary regimens, which restrict energy intake to minimal survival levels, have strong cancer preventive effects in animal models. Observational data reflect a similar effect in humans. Across species, calorie restriction appears to be the most potent, broadly acting dietary regimen for suppressing carcinogenesis (Hursting et al., 2010).

## 4.4. Aging

It is not immediately clear why cancer incidence increases sharply with age, given the evidence (reviewed in section 1), that while cancer driver mutations do accumulate with age, they are not enough alone to initiate cancer. An ecological factor that may explain this trend is that hyperglycemia increases with age, increasing positive selection for mutated neoplastic cells that can use excess glucose for rapid proliferation (Golubev and Anisimov, 2019). Similarly, age-related metabolic shifts which remove resource restrictions have been linked to epithelial cancers (Holly et al., 2013). In general, the process of aging appears to loosen the tightly orchestrated biological controls on tissue nutrition and resource supply, thereby creating intermittent excess.

## 4.5. Micronutrients

Oversupply of limiting cell resources does appear to be an important biological mechanism of cancer risk. This is well-established for energetic macronutrients—the case for micronutrients is less well-established, but also holds potential. The highly-processed foods that now constitute the majority of calories consumed in developed nations not only cause excessive energy intake and tend to increase average daily blood glucose levels (Hall et al., 2019), but also contain additives that can create excessive intake of the limiting micronutrients iron and phosphate. Of all the micronutrients, phosphate may be the most promising candidate for managing cancer risk. Dietary phosphorus deficiency is uncommon and usually observed only in rare inherited disorders, or near-total starvation. There would be little health risk in reducing dietary intake, especially by avoiding the extra phosphate that is an additive in many processed foods and beverages (Erem and Razzaque, 2018). Excess phosphate is implicated in risk of both lung cancer (Jin et al., 2009), and prostate cancer (Perez-Cornago et al., 2020), and high dietary phosphorus intake is associated with all-cause mortality (Chang et al., 2014).

## 4.6. Prevention and Intervention

An excess of limiting cell resources offers excellent opportunities for management of individual cancer risk, because in many cases

it may be measured non-invasively. For example, inexpensive wearable monitors of serum glucose are widely available. Similarly, measurement of urinary phosphate is routinely used to manage kidney disease, and standard test kits might be repurposed for managing cancer risk. In addition to addressing cancer risk, evaluating tissue ecology may also be helpful in the difficult diagnostic task of distinguishing between indolent cancers and clinically aggressive cases that demand immediate treatment (Srivastava et al., 2016). As this association between excess resources and increased cancer risk appears to be robust across cancer subtypes and stages, we expect that ecologically-informed risk management will be an important element of cancer prevention research, and ultimately, of better patient outcomes.

## AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

## ACKNOWLEDGMENTS

The author gives special thanks to John W. Pepper, whose ideas and feedback greatly contributed to this work.

## REFERENCES

Alfarouk, K. O., Ibrahim, M. E., Gatenby, R. A., and Brown, J. S. (2013). Riparian ecosystems in human cancers. *Evol. Appl.* 6, 46–53. doi: 10.1111/eva.12015

Ba, Q., Hao, M., Huang, H., Hou, J., Ge, S., Zhang, Z., et al. (2011). Iron deprivation suppresses hepatocellular carcinoma growth in experimental studies. *Clin. Cancer Res.* 17, 7625–7633. doi: 10.1158/1078-0432.CCR-10-3099

Barrick, J. E., Deatherage, D. E., and Lenski, R. E. (2020). "A test of the repeatability of measurements of relative fitness in the long-term evolution experiment with *Escherichia coli*," in *Evolution in Action: Past, Present and Future* (Springer), 77–89. doi: 10.1007/978-3-030-39831-6_8. Available online at: https://link.springer.com/chapter/10.1007%2F978-3-030-39831-6_8

Beerenwinkel, N., Antal, T., Dingli, D., Traulsen, A., Kinzler, K. W., Velculescu, V. E., et al. (2007). Genetic progression and the waiting time to cancer. *PLoS Comput. Biol.* 3:e225. doi: 10.1371/journal.pcbi.0030225

Blatt, J., Finger, M., Price, V., Crary, S. E., Pandya, A., and Adams, D. M. (2019). Cancer risk in klippel-trenaunay syndrome. *Lymphat. Res. Biol.* 17, 630–636. doi: 10.1089/lrb.2018.0049

Bobko, A. A., Eubank, T. D., Driesschaert, B., Dhimitruka, I., Evans, J., Mohammad, R., et al. (2017). Interstitial inorganic phosphate as a tumor microenvironment marker for tumor progression. *Sci. Rep.* 7:41233. doi: 10.1038/srep41233

Brokaw, J., Katsaros, D., Wiley, A., Lu, L., Su, D., Sochirca, O., et al. (2007). IGF-I in epithelial ovarian cancer and its role in disease progression. *Growth Fact.* 25, 346–354. doi: 10.1080/08977190701838402

Brown, R. B., and Razzaque, M. S. (2018). Phosphate toxicity and tumorigenesis. *Biochim. Biophys. Acta Rev. Cancer* 1869, 303–309. doi: 10.1016/j.bbcan.2018.04.007

Chang, A. R., Lazo, M., Appel, L. J., Gutierrez, O. M., and Grams, M. E. (2014). High dietary phosphorus intake is associated with all-cause mortality: results from Nhanes III. *Am. J. Clin. Nutr.* 99, 320–327. doi: 10.3945/ajcn.113.073148

Chen, Y., Fan, Z., Yang, Y., and Gu, C. (2019). Iron metabolism and its contribution to cancer. *Int. J. Oncol.* 54, 1143–1154. doi: 10.3892/ijo.2019.4720

Choi, J.-Y., Neuhouser, M. L., Barnett, M. J., Hong, C.-C., Kristal, A. R., Thornquist, M. D., et al. (2008). Iron intake, oxidative stress-related genes (MnSOD and MPO) and prostate cancer risk in caret cohort. *Carcinogenesis* 29, 964–970. doi: 10.1093/carcin/bgn056

Collaboration, E. R. F. (2011). Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N. Engl. J. Med.* 364, 829–841. doi: 10.1056/NEJMoa1008862

Da Huang, Y.-S. W., Ye, D.-W., Qi, J., Liu, F., Helfand, B. T., Zheng, S. L., et al. (2020). Prostate volume does not provide additional predictive value to prostate health index for prostate cancer or clinically significant prostate cancer: results from a multicenter study in china. *Asian J. Androl.* 22:539. doi: 10.4103/aja.aja_136_19

Davoodi, S. H., Jamshidi-Naeini, Y., Esmaeili, S., Sohrabvandi, S., and Mortazavian, A. M. (2016). The dual nature of iron in relation to cancer: a review. *Iran. J. Cancer Prevent.* 9:e5494. doi: 10.17795/ijcp-5494

Ducasse, H., Arnal, A., Vittecoq, M., Daoust, S. P., Ujvari, B., Jacqueline, C., et al. (2015). Cancer: An emergent property of disturbed resource-rich environments? Ecology meets personalized medicine. *Evol. Appl.* 8, 527–540. doi: 10.1111/eva.12232

Eaton, S. B., Pike, M. C., Short, R. V., Lee, N. C., Trussell, J., Hatcher, R. A., et al. (1994). Women's reproductive cancers in evolutionary context. *Q. Rev. Biol.* 69, 353–367. doi: 10.1086/418650

Egli, T., and Zinn, M. (2003). The concept of multiple-nutrient-limited growth of microorganisms and its application in biotechnological processes. *Biotechnol. Adv.* 22, 35–43. doi: 10.1016/j.biotechadv.2003.08.006

Erem, S., and Razzaque, M. S. (2018). Dietary phosphate toxicity: an emerging global health concern. *Histochem. Cell Biol.* 150, 711–719. doi: 10.1007/s00418-018-1711-8

Feinmesser, M., Taube, E., Badani, E., and Kristt, D. (1997). Basal cell carcinomas arising over arteriovenous malformations: some speculations on the theme. *Am. J. Dermatopathol.* 19, 575–579. doi: 10.1097/00000372-199712000-00004

Fortunato, A., Boddy, A., Mallo, D., Aktipis, A., Maley, C. C., and Pepper, J. W. (2017). Natural selection in cancer biology: from molecular snowflakes to trait hallmarks. *Cold Spring Harb. Perspect. Med.* 7:a029652. doi: 10.1101/cshperspect.a029652

Franceschini, G., Lello, S., and Masetti, R. (2020). Hormone replacement therapy: revisiting the risk of breast cancer. *Eur. J. Cancer Prevent.* 29, 303–305. doi: 10.1097/CEJ.0000000000000548

Gillies, R. J., Robey, I., and Gatenby, R. A. (2008). Causes and consequences of increased glucose metabolism of cancers. *J. Nucl. Med.* 49:24S. doi: 10.2967/jnumed.107.047258

Giovannucci, E. (1999). "Nutritional factors in human cancers," in *Advances in Nutrition and Cancer 2* (Boston, MA: Springer), 29–42. doi: 10.1007/978-1-4757-3230-6_3. Available online at: https://link.springer.com/chapter/10.1007%2F978-1-4757-3230-6_3

Golubev, A. G., and Anisimov, V. N. (2019). Aging and cancer: is glucose a mediator between them? *Oncotarget* 10:6758. doi: 10.18632/oncotarget.27344

Hall, K. D., Ayuketah, A., Brychta, R., Cai, H., Cassimatis, T., Chen, K. Y., et al. (2019). Ultra-processed diets cause excess calorie intake and weight gain: an inpatient randomized controlled trial of ad libitum food intake. *Cell Metab.* 30, 67–77. doi: 10.1016/j.cmet.2019.05.008

Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* 100, 57–70. doi: 10.1016/S0092-8674(00)81683-9

Holly, J. M., Biernacka, K., and Perks, C. M. (2019). Systemic metabolism, its regulators, and cancer: past mistakes and future potential. *Front. Endocrinol.* 10:65. doi: 10.3389/fendo.2019.00065

Holly, J. M., Zeng, L., and Perks, C. M. (2013). Epithelial cancers in the post-genomic era: should we reconsider our lifestyle? *Cancer Metast. Rev.* 32, 673–705. doi: 10.1007/s10555-013-9445-5

Hursting, S. D., and Berger, N. A. (2010). Energy balance, host-related factors, and cancer progression. *J. Clin. Oncol.* 28:4058. doi: 10.1200/JCO.2010.27.9935

Hursting, S. D., Smith, S. M., Lashinger, L. M., Harvey, A. E., and Perkins, S. N. (2010). Calories and carcinogenesis: lessons learned from 30 years of calorie restriction research. *Carcinogenesis* 31, 83–89. doi: 10.1093/carcin/bgp280

Jin, H., Xu, C.-X., Lim, H.-T., Park, S.-J., Shin, J.-Y., Chung, Y.-S., et al. (2009). High dietary inorganic phosphate increases lung tumorigenesis

and alters AKT signaling. *Am. J. Respir. Crit. Care Med*. 179, 59–68. doi: 10.1164/rccm.200802-306OC

Kalaany, N. Y., and Sabatini, D. M. (2009). Tumours with pi3k activation are resistant to dietary restriction. *Nature* 458, 725–731. doi: 10.1038/nature07782

Lapidoth, M., Ad-El, D., David, M., Alcalay, J., and Azaria, R. (2006). Basal cell carcinoma arising over facial port wine stain: a single-centre experience. *J. Eur. Acad. Dermatol. Venereol*. 20, 1066–1069. doi: 10.1111/j.1468-3083.2006.01701.x

Lenski, R. E. (2017). Convergence and divergence in a long-term experiment with bacteria. *Am. Nat*. 190, S57–S68. doi: 10.1086/691209

Lloyd, M. C., Alfarouk, K. O., Verduzco, D., Bui, M. M., Gillies, R. J., Ibrahim, M. E., et al. (2014). Vascular measurements correlate with estrogen receptor status. *BMC Cancer* 14:279. doi: 10.1186/1471-2407-14-279

Louro, J., Posso, M., Boon, M. H., Román, M., Domingo, L., Castells, X., et al. (2019). A systematic review and quality assessment of individualised breast cancer risk prediction models. *Br. J. Cancer* 121, 76–85. doi: 10.1038/s41416-019-0476-8

Maley, C. C., Aktipis, A., Graham, T. A., Sottoriva, A., Boddy, A. M., Janiszewska, M., et al. (2017). Classifying the evolutionary and ecological features of neoplasms. *Nat. Rev. Cancer* 17, 605–619. doi: 10.1038/nrc.2017.69

Maley, C. C., Szabo, E., and Reid, B. J. (2011). "Somatic evolution in neoplastic progression and cancer prevention," in *Pre-Invasive Disease: Pathogenesis and Clinical Management* (Springer), 111–127. doi: 10.1007/978-1-4419-6694-0_7

Marrone, M. T., Selvin, E., Barber, J. R., Platz, E. A., and Joshu, C. E. (2019). Hyperglycemia, classified with multiple biomarkers simultaneously in men without diabetes, and risk of fatal prostate cancer. *Cancer Prevent. Res*. 12, 103–112. doi: 10.1158/1940-6207.CAPR-18-0216

Muti, P., Quattrin, T., Grant, B. J., Krogh, V., Micheli, A., Schünemann, H. J., et al. (2002). Fasting glucose is a risk factor for breast cancer: a prospective study. *Cancer Epidemiol. Prevent. Biomark*. 11, 1361–1368.

Nam, A. S., Chaligne, R., and Landau, D. A. (2020). Integrating genetic and non-genetic determinants of cancer evolution by single-cell multi-omics. *Nat. Rev. Genet*. 22, 3–18. doi: 10.1038/s41576-020-0265-5

Navarro, J., Kang, I., Hwang, H. K., Yoon, D. S., Lee, W. J., and Kang, C. M. (2019). Glucose to lymphocyte ratio as a prognostic marker in patients with resected PT2 gallbladder cancer. *J. Surg. Res*. 240, 17–29. doi: 10.1016/j.jss.2019.02.043

Pati, D., and Lorusso, L. N. (2018). How to write a systematic review of the literature. *Health Environ. Res. Des. J*. 11, 15–30. doi: 10.1177/1937586717747384

Perez-Cornago, A., Fensom, G. K., Andrews, C., Watts, E. L., Allen, N. E., Martin, R. M., et al. (2020). Examination of potential novel biochemical factors in relation to prostate cancer incidence and mortality in UK biobank. *Br. J. Cancer* 123, 1808–1817. doi: 10.1038/s41416-020-01081-3

Reynolds, B. A., Oli, M. W., and Oli, M. K. (2020). Eco-oncology: applying ecological principles to understand and manage cancer. *Ecol. Evol*. 10, 8538–8553. doi: 10.1002/ece3.6590

Richmond, H. (1959). Induction of sarcoma in the rat by iron-dextran complex. *Br. Med. J*. 1:947. doi: 10.1136/bmj.1.5127.947

Shapiro, B., Hoehler, T. M., and Jin, Q. (2018). Integrating genome-scale metabolic models into the prediction of microbial kinetics in natural environments. *Geochim. Cosmochim. Acta* 242, 102–122. doi: 10.1016/j.gca.2018.08.047

Solary, E., and Laplane, L. (2020). The role of host environment in cancer evolution. *Evol. Appl*. 13, 1756–1770. doi: 10.1111/eva.13039

Srivastava, S., Reid, B. J., Ghosh, S., and Kramer, B. S. (2016). Research needs for understanding the biology of overdiagnosis in cancer screening. *J. Cell. Physiol*. 231, 1870–1875. doi: 10.1002/jcp.25227

Tomasetti, C. (2019). Mutated clones are the new normal. *Science* 364, 938–939. doi: 10.1126/science.aax5525

Torti, S., Torti, F., Whitman, S., Brechbiel, M., Park, G., and Planalp, R. (1998). Tumor cell cytotoxicity of a novel metal chelator. *Blood* 92, 1384–1389. doi: 10.1182/blood.V92.4.1384.416k30_1384_1389

Torti, S. V., and Torti, F. M. (2013). Iron and cancer: more ore to be mined. *Nat. Rev. Cancer* 13, 342–355. doi: 10.1038/nrc3495

Watts, E. L., Perez-Cornago, A., Appleby, P. N., Albanes, D., Ardanaz, E., Black, A., et al. (2019). The associations of anthropometric, behavioural and sociodemographic factors with circulating concentrations of IGF-I, IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3 in a pooled analysis of 16,024 men from 22 studies. *Int. J. Cancer* 145, 3244–3256. doi: 10.1002/ijc.32276

Weinberg, E. (1996). The role of iron in cancer. *Eur. J. Cancer Prevent*. 5, 19–36.

Weinberg, E. D. (1984). Iron withholding: a defense against infection and neoplasia. *Physiol. Rev*. 64, 65–102. doi: 10.1152/physrev.1984.64.1.65

Whitnall, M., Howard, J., Ponka, P., and Richardson, D. R. (2006). A class of iron chelators with a wide spectrum of potent antitumor activity that overcomes resistance to chemotherapeutics. *Proc. Natl. Acad. Sci. U.S.A*. 103, 14901–14906. doi: 10.1073/pnas.0604979103

Wu, D. J., Aktipis, A., and Pepper, J. W. (2019). Energy oversupply to tissues: a single mechanism possibly underlying multiple cancer risk factors. *Evol. Med. Publ. health* 2019, 9–16. doi: 10.1093/emph/eoz004

Yamamoto, N., Oshima, T., Yoshihara, K., Aoyama, T., Hayashi, T., Yamada, T., et al. (2017). Clinicopathological significance and impact on outcomes of the gene expression levels of IGF-1, IGF-2 and IGF-1R, IGFBP-3 in patients with colorectal cancer: overexpression of the IGFBP-3 gene is an effective predictor of outcomes in patients with colorectal cancer. *Oncol. Lett*. 13, 3958–3966. doi: 10.3892/ol.2017.5936

Yang, I.-P., Tsai, H.-L., Huang, C.-W., Lu, C.-Y., Miao, Z.-F., Chang, S.-F., et al. (2016). High blood sugar levels significantly impact the prognosis of colorectal cancer patients through down-regulation of microRNA-16 by targeting MYB and VEGFR2. *Oncotarget* 7:18837. doi: 10.18632/oncotarget.7719

Zhang, Y., Xu, Y., Wang, D., Kuang, T., Wu, W., Xu, X., et al. (2020). Prognostic value of preoperative glucose to lymphocyte ratio in patients with resected pancreatic cancer. *Int. J. Clin. Oncol*. 26, 135–144. doi: 10.1007/s10147-020-01782-y

**Conflict of Interest:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

PERSPECTIVE
published: 20 May 2021
doi: 10.3389/fevo.2021.666262

# Testing Multi-Task Cancer Evolution: How Do We Test Ecological Hypotheses in Cancer?

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Recently several authors described a family of models, according to which different cancer types and subtypes fall within a space of selective trade-offs between archetypes that maximize the performance of different tasks: cell division, biomass and energy production, lipogenesis, immune interaction, and invasion and tissue remodeling. On this picture, inter- and intratumor heterogeneity can be explained in part as a product of these selective trade-offs in different cancers, at different stages of cancer progression. The aim of this Perspective is to critically assess this approach. I use this case study to consider more generally both the advantages of using ecological models in the context of cancer, and the challenges facing testing of such models.

**Keywords: multi-task, evolution, cancer, ecology, testing, genomics**

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***Edited by:***
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***Specialty section:***
*This article was submitted to Models in Ecology and Evolution, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *09 February 2021*
***Accepted:*** *23 April 2021*
***Published:*** *20 May 2021*

***Citation:***
*Plutynski A (2021) Testing Multi-Task Cancer Evolution: How Do We Test Ecological Hypotheses in Cancer? Front. Ecol. Evol. 9:666262. doi: 10.3389/fevo.2021.666262*

## INTRODUCTION

Cancer evolves; that is, populations of cancer cells change over time in distribution of genotypic and phenotypic features, and relative survival is due in part to interactions with the surrounding environment. This idea is not new, and has indeed led to an active research program (Nowell, 1976; Merlo et al., 2006; Greaves and Maley, 2012). If cancers evolve, then investigating the *ecologies* of cancers, and selective trade-offs at work in these different local microenvironments, will be centrally important to explaining how and why cancers progress slowly or quickly, respond to treatment, or fail to do so.

What, however, does it mean to explain or describe cancer's "ecologies" or "ecological dynamics"? While several scientists have proposed general theoretical frameworks and mathematical models for predicting and explaining cancer's evolutionary dynamics (Michor et al., 2004; Frank, 2007; Wodarz and Komarova, 2014), relatively few have drawn upon ecological theory (F. Adler and Gordon, 2019). However, Maley et al. (2017) describe what they call the "Evo-" and "Eco-Index" of cancers – that is, a taxonomy of various features that enable various patterns of evolutionary and ecological change in cancers over time. Thus, for instance, a major component of the "Evo-index" of a tumor is *extent of heterogeneity*, which enables a population of cancer cells to respond to selection. The "Eco-Index," in contrast, consists in a "profile" of "hazards" and "resources" (what can kill a cell, or resources required for cell maintenance and growth), which might be expected to select for the particular life history strategies (Aktipis et al., 2013). High levels of hazard or fluctuating resources might tend to yield rapid reproduction and little investment in maintenance and survival. Low hazards and a steady supply of resources, in contrast, might predict an expansion of the carrying capacity of the habitat, and competition for limiting resources.

International sequencing efforts have now provided data that allows cancer researchers to test some of these hypotheses (Hutter and Zenklusen, 2018). These sequencing efforts demonstrated that cancers are enormously heterogeneous. Cancers arising in different cell types, tissues, or organs vary in the extent and type of mutations most common. This "inter-tumor" heterogeneity is often contrasted with "intra-tumor" heterogeneity: the extent of genetic variation within a single population of cancer cells. In order to optimize treatment, we need to better understand why variation arises between cancers, and among cell lineages in a tumor, both in space, and over time.

Hausser and Alon (2020) apply multitask evolution to genomic data, in service of identifying the specific trade-offs at work in different cancers, and among cell lineages (Hausser et al., 2019, p. 2). They predicted that, given trade-offs among various tasks of cancer cells in a tumor, both space, and over time, selection among these trade-offs could also yield "archetypal" genomic profiles. For instance, early on in the development of a tumor, one might expect genetic profiles associated with rapid growth, whereas later on, there may be genetic profiles associated with immune resistance, or capacity for invasion and metastasis.

To identify the trade-offs at work in cancer progression, they use a Pareto-optimal modeling strategy, drawing upon gene expression profile data (transcriptomic data) from TCGA and METABRIC databases. Using PCA (principal component analysis), they reduced the number of dimensions in the data, identifying the most common variants across tumors. They then subject this reduced dataset to ParTI (Pareto task inference). ParTI has been used to illustrate the role of selective trade-offs between tasks in a variety of other systems. The "Pareto front" represents gene expression profiles for which performance cannot be improved without decreasing performance in another task: gene expression profiles along a Pareto front are "Pareto optimal." When there are three or more tasks, one can generate a polyhedron, where the vertices represent the "archetypes" – or, "specialists" at specific tasks.

They showed that different cancers seem to have distinctive optima, or gene expression profiles associated with trade-offs among different tasks. For instance, in glioma, the trade-offs were between cell division, invasion and tissue remodeling, and immune interaction, with a cluster close to the cell division archetype. In contrast, in liver cancer, the trade-offs appear to be between biomass and energy production, cell division, and invasion and metastasis, with a cluster closer to biomass and energy production. Moreover, they found that, depending upon stage or grade, different cancers within a type (e.g., breast cancers) seemed to display gene expression of higher frequency coinciding with one or another Pareto optimum, suggesting that selective trade-offs likely change from early stage tumors to invasive metastatic disease. Such selective trade-offs might be driving change in the distribution of tasks in cell populations in a tumor over time, and thus, changes in the distribution of gene expression profiles. Such information could, they argue, be linked with clinical data, and drug sensitivity data, in service of more effective therapy.

There were some limitations to their analysis, however. They "could not reliably detect polyhedra for seven out of 15 cancer types; these seven cancer types showed gene expression that fell in a cloud without detectable vertices." That is, fully half of the cancer types they analyzed did not fall within the archetype framework. As they note, future research could determine what might explain lack of fit, where one option is simply that "trade-off theory is not applicable such as a lack of strong selection," or, perhaps, "too many tasks (many archetypes) that cannot be resolved given the noise." (Hausser and Alon, 2020, 250) Below, this example will be considered as a case study for generating important insights about what we ought to look for when testing hypotheses about cancer's eco-evolutionary dynamics.

## CANCER GENOMIC DATA AS A SOURCE OF BIAS

Hausser et al., generated their archetypes by drawing upon TCGA and METABRIC data, reducing the dimensions of the data using PCA (principal component analysis). It's worth briefly considering how these data were generated, to consider whether either the data themselves, or the reduction in of dimensions of the data (or both), might bias the results they found.

The TCGA "pipeline" had several stages. First, tumor samples and healthy cells are taken from each patient, typically at first diagnosis – i.e., early stage cancers. Though, how early this may have been in the progression of disease likely varied significantly across cancers – for instance, pancreatic cancers tend to be diagnosed later than prostate or breast cancers. Second, at least during the first 5 years during which TCGA was conducted, whole exome sequencing was not an option. So, initially, the second stage of the pipeline involved targeted sequencing of genes known (already) to be tumor drivers: genes, mutation to which were already known to be common in cancers of this or that type (Hutter and Zenklusen, 2018). The third stage involved comparing frequency of different mutations within cancers of a particular type or subtype. During the last half decade of sequencing efforts, whole exome sequencing and "mutation calling" algorithms, systematically generated data on which mutations were common or rare in different cancer types. These algorithms were designed to exclude certain genes not known or believed to be relevant to cancer phenotype, and thus *weighted some genes as likely more significant than others*, based on functions known or likely typically associated with the cancer phenotype – e.g., if a gene was associated with mitosis, etc.

In other words, the driver mutations identified by TCGA as more or less common in cancers of this or that type were identified by algorithms designed to detect mutations to genes known to be associated "hallmark" functions of cancer cells (e.g., TP53, APC, etc.) (Hanahan and Weinberg, 2011). Genes typically thought to have no role in "hallmark" features of cancer cells were (by and large) ruled out as "noise." Thus, one concern that any analysis of cancer genomic data may have when using such data to test hypotheses about selective trade-offs is that cancer genomic data (at least that data published in the consensus genome papers) were already filtered by algorithms designed to identify mutations to genes associated with the "hallmarks" of cancer. Thus, it is no surprise that data drawn from TCGA

would generate "archetypes," or show relatively high frequency of mutation and/or gene expression for these five major tasks. Hausser and Alon's (2020) discovery that glioma and bladder tumors, for instance, fit a polyhedron model, with axes that are represent trade-offs among gene expression for specialization in cell division or immune interaction, in other words, may in part be not entirely unexpected. Further, their analysis reduced the dimensions of the data, and averaged gene expression patterns within any given cancer type or subtype – such a process may have led to loss of important information, such as about unique gene expression profiles distinctive to particular cancers, or subpopulations of cells within cancers. This could well explain the lack of fit with the models, for a proportion of the cancers studied.

## STANDARDS OF EVIDENCE IN TESTING ECOLOGICAL MODELS IN CANCER

A second general concern one might have has to do with standards of evidence for testing hypotheses about trade-offs. In a classic discussion, Stearns (1989) gives a brief overview of what information is required to test hypotheses about life history trade-offs in whole organism biology:

That trade-offs can be measured and analyzed at the level of the genotype, the phenotype and what lies between (intermediate structure) . . . *It is not a question of either genetic correlations or phenotypic correlations or physiological trade-offs but of how such measurements combine to deliver information about potential evolutionary responses*. A study conducted at just one of these levels is likely to be of as little use as the information on the nature of the elephant delivered by one blind man holding its tail ... Knowledge of all three of these levels is necessary to understand how a trade-off works (Stearns, 1989, p. 259).

Stearns gives several examples of tests of hypotheses about life-history trade-offs – for instance, trading off between growth and reproduction. In all these models, there is a quantitative measure of the traits in question in a given population, their effects on fitness, and in some cases, experimental manipulation of the population to test these hypotheses.

According to Stearns, for a genuine test of an ecological hypothesis about trade-offs, it is important to give quantitative measures of how trade-offs between phenotypic traits negatively covary. Moreover, in principle, one should also establish that there was sufficient variation within the initial population for both traits to be subject to selection. If manipulation of the traits is possible, experimental manipulations should be conducted to test hypotheses about these proposed trade-offs. Ideally one must give ecological information about how and why traits are likely to trade off, and not only demonstrate how they negatively covary. Testing requires some quantitative measure of fitness, a function that describes how fitness depends on variable phenotypes (and trade-offs among them), and a set of alternative phenotypic profiles that describes options for manipulating the variables at work in these fitness trade-offs. How does Hausser et al.'s theoretical framework perform in this regard?

They do cite indirect evidence that there are plausibly selective trade-offs likely at work in cancer. Some resources, such as ATP, are needed for both growth and metastasis, and are limited in supply (Broxterman et al., 1988), metabolic constraints were also reported (Jerby et al., 2012), harsh conditions cause cancer cells to become quiescent (Gade et al., 2017), and proliferation is stimulated more favorable microenvironments (Wang et al., 2017a,b). Hausser et al., cite several papers that they claim support the general view that cancer cells face fitness trade-offs (Hatzikirou et al., 2012; Aktipis et al., 2013; Gillies et al., 2018; Gallaher et al., 2019).

However, a closer look at these papers indicates that they show not that cancer cells do as a matter of fact face trade-offs between various traits in a given environment, but only that this is a plausible hypothesis. For instance, Aktipis et al. (2013) write, "The exact nature of tradeoffs between these mechanisms has yet to be determined in most cases." Gallaher et al.'s (2019) is an ingenious simulation, using agent-based modeling to represent how these trade-offs could evolve in a population of cancer cells. However, the paper presupposes, rather than documents, the trade-offs in question. Likewise, Gillies et al. (2018), discussion is about how it is plausible that various trade-offs are at play in the EMT (epithelial-mesenchymal transition), associated with changes in blood flow in the tumor, not a test of this hypothesis. While they provide evidence suggesting that this hypothesis is a plausible explanation of patterns and processes of changes in tumors, it is not an attempt at systematically testing the hypothesis. Hatzikirou et al. (2012), also cite experiments with cultures of glioma cells (Giese et al., 2003) that have shown a "relationship between migratory and proliferative behavior, indicating cell motion and proliferation are mutually exclusive processes since highly motile glioma cells tend to have lower proliferation rates." (Giese et al., 1996a,b; Godlewski et al., 2010). However, the Hatzikirou et al. (2012) do not themselves conduct any experiments; the paper is *simulation of how the trade-off is likely* to play out in glioma. So, such studies do not provide the kinds of tests of life-history trade-offs Stearns takes to be exemplary; much of the evidence is indirect, at best.

On the one hand, one might argue that holding cancer researchers to the same standards of testing trade-offs typical in whole organism ecology is inappropriate. After all, cancers are often discovered well after the selective processes in question occurred. Unlike in whole organism biology and ecology, we cannot do a controlled study of exactly how and how much cancer cells vary with respect to these trade-offs *in situ*. Simulations are as close to tests of such hypotheses as can be provided (Parke, 2014). In the best case scenario, and perhaps with advances in sampling of tumor biomarkers, we may be able to describe the dynamics of cancer's evolution, during the course of treatment. Just as in testing any evolutionary hypotheses for which the evidence is long in the past, we can use experimental or computer simulations of *close enough* evolutionary processes (Vasi et al., 1994; Sniegowski et al., 1997).

On the other hand, it does seem worth considering whether ecological models and evidence in cancer should be held to lesser standards of. In order to test hypotheses that selective trade-offs are at work, or that various optima explain the presence or absence of this variant distribution in a population, whole organism ecologists are typically expected to generate a

*function* relating fitness and variable phenotypes (and trade-offs among them), and describe a *how* these fitness trade-offs can be varied to yield quantitative differences in outcome. Hausser and Alon (2020) do not provide anything this precise, nor do they experimentally test the link by manipulating these variables. Determining whether such fitness trade-offs are at work might require more precise, quantitative measures. Such context-specific information may be rather important to have, especially in treatment contexts.

Indeed, as Hausser et al. suggest, local selective (i.e., ecological) conditions may vary significantly across cancers. Arguably, different tumor microenvironments present quite distinctive challenges, and thus different selective "tasks" for different cancers, and different trade-offs, other than those they consider. It seems one important avenue for future work is to consider more seriously the role of local ecology – and potentially also, a role for niche construction. While it seems plausible that cancer cells from a variety of tissues and organs have relatively similar "driver" or hallmark gene expression profiles, it's also plausible that local conditions vary significantly (cf., Pong and Gutmann, 2011).

## CONCLUSION

Multitask evolutionary theory is potentially a quite fruitful theoretical framework for generating and testing hypotheses that may explain the massive heterogeneity within and across cancer types and subtypes. It seems plausible, as Hausser et al., argue, that a variety of selection processes, and thus fitness optima, are universal to all cancers, and that there are trade-offs among various gene expression profiles. However, a significant portion of the cancers Hausser et al. studied did not fall within the archetypal framework. There are many possible explanations – ranging from the way the data were generated, to the means of analysis. I've argued here that it is worth exploring how cancers' dynamics might be governed by different ecological conditions, in different tissue microenvironments. Another consideration is drift; selection optimizes only given sufficient variation to act upon. Drift may play a significant role in some cancers' dynamics, limiting variation available for selection. Cancer stem cells may effectively function as genetic "bottlenecks," governing the variation available for selection in a tumor (Laplane, 2018; Lyne et al., 2020). Such bottlenecks could be limiting the possible scope of evolutionary change in some cancers.

I've also described two other reasons to be cautious in interpreting their results in light of the data used. When we set up an analysis of genomic data, we should be careful to assess whether the options are "forced" by the data or model considered. There are two ways in which this forcing could have come about here; first, their framework required that cancer types or subtypes be subject to trade-offs in ways that force the choice between "generalists" or "specialists." Second, the "tasks" that they identify were arguably "baked in": they are the very same tasks that cancer genomics researchers have been seeking to link to cancer drivers: the "hallmarks" of cancer. That said, it's not implausible that different cancer types in very different tissue microenvironments have distinctive ecological conditions, and thus selective trade-offs, at play.

Life history trade-off hypotheses may be easy to develop, but tests of such hypotheses can be forbiddingly difficult to carry out. As attested by Stearns (1989), examples of successful tests in whole organism biology often required decades of field work and experimental manipulation. On the one hand, it is widely agreed that life history theory, hypotheses about adaptation to local environments, and adaptive optima, can be fruitful. On the other hand, to establish exactly how various trade-offs are at work (such as a limited supply of energy, time, biomass, or nutrients), we should in principle give quantitative measures of each traits' relative effects on fitness. Even better, we should demonstrate how they change over time, drawing upon some form of experimental manipulation. Please see the attached figure for a summary of key parameters of relevance to testing hypotheses about ecological trade-offs in cancer.

## AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.666262/full#supplementary-material

## REFERENCES


Adler, F. R., and Gordon, D. M. (2019). Cancer ecology and evolution: positive interactions and system vulnerability. *Curr. Opin. Syst. Biol.* 17, 1–7. doi: 10.1016/j.coisb.2019.09.001

Adler, M., Kohanim, Y. K., Tendler, A., Mayo, A., and Alon, U. (2019). Continuum of gene-expression profiles provides spatial division of labor within a differentiated cell type. *Cell Syst.* 8, 43–52. doi: 10.1016/j.cels.2018.12.008

Aktipis, C. A., Boddy, A. M., Gatenby, R. A., Brown, J. S., and Maley, C. C. (2013). Life history trade-offs in cancer evolution. *Nat. Rev. Cancer* 13, 883–892. doi: 10.1038/nrc3606

Broxterman, H. J., Pinedo, H. M., Kuiper, C. M., Kaptein, L. C., Schuurhuis, G. J., and Lankelma, J. (1988). Induction by verapamil of a rapid increase in ATP consumption in multidrugresistant tumor cells. *FASEB J.* 2, 2278–2282. doi: 10.1096/fasebj.2.7.3350243

Frank, S. A. (2007). *Dynamics of Cancer: Incidence, Inheritance, and Evolution*. Princeton, NJ: Princeton University Press. doi: 10.1515/9780691186863

Gade, T. P. F., Tucker, E., Nakazawa, M. S., Hunt, S. J., Wong, W., Krock, B., et al. (2017). Ischemia induces quiescence and autophagy dependence in hepatocellular carcinoma. *Radiology* 283, 702–710. doi: 10.1148/radiol.2017160728

Gallaher, J. A., Brown, J. S., and Anderson, A. R. A. (2019). The impact of proliferation- migration tradeoffs on phenotypic evolution in cancer. *Sci. Rep.* 9, 1–10. doi: 10.1038/s41598-019-39636-x

Giese, A., Bjerkvig, R., Berens, M. E., and Westphal, M. (2003). Cost of migration: invasion of malignant gliomas and implications for treatment. *J. Clin. Oncol.* 21, 1624–1636. doi: 10.1200/JCO.2003.05.063

Giese, A., Kluwe, L., Laube, B., Meissner, H., Berens, M. E., and Westphal, M. (1996b). Migration of human glioma cells on myelin. *Neurosurg.* 38, 755–764. doi: 10.1227/00006123-199604000-00026

Giese, A., Loo, M. A., Tran, N., Haskett, D., Coons, S. W., and Berens, M. E. (1996a). Dichotomy of astrocytoma migration and proliferation. *Int. J. Cancer* 67, 275–282. doi: 10.1002/(SICI)1097-0215(19960717)67:2<275::AID-IJC20>3.0.CO;2-9

Gillies, R. J., Brown, J. S., Anderson, A. R. A., and Gatenby, R. A. (2018). Eco-evolutionary causes and consequences of temporal changes in intratumoural blood flow. *Nat. Rev. Cancer* 18, 576–585. doi: 10.1038/s41568-018-0030-7

Godlewski, J., Bronisz, A., Nowicki, M. O., Chiocca, E. A., and Lawler, S. (2010). microRNA-451: a conditional switch controlling glioma cell proliferation and migration. *Cell Cycle* 9, 2814–2820. doi: 10.4161/cc.9.14.12248

Greaves, M., and Maley, C. C. (2012). Clonal evolution in cancer. *Nature* 481, 306–313. doi: 10.1038/nature10762

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Hatzikirou, H., Basanta, D., Simon, M., Schaller, K., and Deutsch, A. (2012). 'Go or grow': the key to the emergence of invasion in tumour progression? *Math. Med. Biol.* 29, 49–65. doi: 10.1093/imammb/dqq011

Hausser, J., and Alon, U. (2020). Tumour heterogeneity and the evolutionary trade-offs of cancer. *Nat. Rev. Cancer* 20, 247–257. doi: 10.1038/s41568-020-0241-6

Hausser, J., Szekely, P., Bar, N., Zimmer, A., Sheftel, H., and Caldas, C. (2019). Tumor diversity and the trade-off between universal cancer tasks. *Nat. Commun.* 10:5423. doi: 10.1038/s41467-019-13195-1

Hutter, C., and Zenklusen, J. C. (2018). The cancer genome atlas: creating lasting value beyond its data. *Cell* 173, 283–285. doi: 10.1016/j.cell.2018.03.042

Jerby, L., Wolf, L., Denkert, C., Stein, G. Y., Hilvo, M., Oresic, M., et al. (2012). Metabolic associations of reduced proliferation and oxidative stress in advanced breast cancer. *Cancer Res.* 72, 5712–5720. doi: 10.1158/0008-5472.CAN-12-2215

Laplane, L. (2018). Cancer stem cells modulate patterns and processes of evolution in cancers. *Biol. Philos.* 33, 1–25. doi: 10.1007/s10539-018-9629-z

Lyne, A. M., Laplane, L., and Perié, L. (2020). To portray clonal evolution in blood cancer, count your stem cells. *Blood* 137, 1862–1870. doi: 10.1182/blood.2020008407

Maley, C. C., Aktipis, A., Graham, T. A., Sottoriva, A., Boddy, A. M., Janiszewska, M., et al. (2017). Classifying the evolutionary and ecological features of neoplasms. *Nat. Rev. Cancer* 17:605. doi: 10.1038/nrc.2017.69

Merlo, L. M. F., Pepper, J. W., Reid, B. J., and Maley, C. C. (2006). Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer* 6, 924–935. doi: 10.1038/nrc2013

Michor, F., Iwasa, Y., and Nowak, M. A. (2004). Dynamics of cancer progression. *Nat. Rev. Cancer* 4, 197–205. doi: 10.1038/nrc1295

Nowell, P. C. (1976). The clonal evolution of tumor cell populations. *Science* 194, 23–28. doi: 10.1126/science.959840

Parke, E. C. (2014). Experiments, simulations, and epistemic privilege. *Philos. Sci.* 81, 516–536. doi: 10.1086/677956

Pong, W. W., and Gutmann, D. H. (2011). The ecology of brain tumors: lessons learned from neurofibromatosis-1. *Oncogene* 30, 1135–1146. doi: 10.1038/onc.2010.519

Sniegowski, P. D., Gerrish, P. J., and Lenski, R. E. (1997). Evolution of high mutation rates in experimental populations of *E. coli*. *Nature* 387, 703–705. doi: 10.1038/42701

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Funct. Ecol.* 3, 259–268. doi: 10.2307/2389364

Vasi, F., Travisano, M., and Lenski, R. E. (1994). Long-term experimental evolution in *Escherichia coli*. II. Changes in life-history traits during adaptation to a seasonal environment. *Am. Nat.* 144, 432–456. doi: 10.1086/285685

Wang, X., Fujimaki, K., Mitchell, G. C., Kwon, J. S., Della Croce, K., Langsdorf, C., et al. (2017a). Exit from quiescence displays a memory of cell growth and division. *Nat. Commun.* 8:321. doi: 10.1038/s41467-017-00367-0

Wang, Y. K., Bashashati, A., Anglesio, M. S., Cochrane, D. R., Grewal, D. S., Ha, G., et al. (2017b). Genomic consequences of aberrant DNA repair mechanisms stratify ovarian cancer histotypes. *Nat. Genet.* 49, 856–865.

Wodarz, D., and Komarova, N. (2014). *Dynamics of Cancer: Mathematical Foundations of Oncology*. Toh Tuck: World Scientific. doi: 10.1142/8973

**Conflict of Interest:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 14 June 2021
doi: 10.3389/fevo.2021.675800

# Hypoxia Generated by Avian Embryo Growth Induces the HIF-α Response and Critical Vascularization

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OPEN ACCESS

**Edited by:**
*Frederick R. Adler, The University of Utah, United States*

**Reviewed by:**
*Gerardo Antonio Cordero, University of Tübingen, Germany*
*Jeffrey West, Moffitt Cancer Center, United States*

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**Specialty section:**
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

**Received:** *03 March 2021*
**Accepted:** *03 May 2021*
**Published:** *14 June 2021*

**Citation:**
*Carroll C, Engström N, Nilsson PF, Haxen ER, Mohlin S, Berg P, Glud RN and Hammarlund EU (2021) Hypoxia Generated by Avian Embryo Growth Induces the HIF-α Response and Critical Vascularization. Front. Ecol. Evol. 9:675800. doi: 10.3389/fevo.2021.675800*

Cancer research has transformed our view on cellular mechanisms for oxygen sensing. It has been documented that these mechanisms are important for maintaining animal tissues and life in environments where oxygen ($O_2$) concentrations fluctuate. In adult animals, oxygen sensing is governed by the Hypoxia Inducible Factors (HIFs) that are stabilized at low oxygen concentrations (hypoxia). However, the importance of hypoxia itself during development and for the onset of HIF-driven oxygen sensing remains poorly explored. Cellular responses to hypoxia associates with cell immaturity (stemness) and proper tissue and organ development. During mammalian development, the initial uterine environment is hypoxic. The oxygenation status during avian embryogenesis is more complex since $O_2$ continuously equilibrates across the porous eggshell. Here, we investigate HIF dynamics and use microelectrodes to determine $O_2$ concentrations within the egg and the embryo during the first four days of development. To determine the increased $O_2$ consumption rates, we also obtain the $O_2$ transport coefficient ($D_{O2}$) of eggshell and associated inner and outer shell membranes, both directly (using microelectrodes *in ovo* for the first time) and indirectly (using water evaporation at 37.5°C for the first time). Our results demonstrate a distinct hypoxic phase (<5% $O_2$) between day 1 and 2, concurring with the onset of HIF-α expression. This phase of hypoxia is demonstrably necessary for proper vascularization and survival. Our indirectly determined $D_{O2}$ values are about 30% higher than those determined directly. A comparison with previously reported values indicates that this discrepancy may be real, reflecting that water vapor and $O_2$ may be transported through the eggshell at different rates. Based on our obtained $D_{O2}$ values, we demonstrate that increased $O_2$ consumption of the growing embryo appears to generate the phase of hypoxia, which is also facilitated by the initially small gas cell and low membrane permeability. We infer that the phase of *in ovo* hypoxia facilitates correct avian development. These results support

the view that hypoxic conditions, in which the animal clade evolved, remain functionally important during animal development. The study highlights that insights from the cancer field pertaining to the cellular capacities by which both somatic and cancer cells register and respond to fluctuations in $O_2$ concentrations can broadly inform our exploration of animal development and success.

**Keywords: hypoxia, embryogenesis, eggshell membrane, diffusion coefficient, oxygen consumption rates ($V_{O2}$), evolution, HIF-α, cancer**

## HIGHLIGHTS

- $O_2$ and HIF dynamics *in ovo* and in tissue during day 0–4 of avian embryogenesis.
- Early phase of *in ovo* hypoxia, despite the eggshell being permeable to $O_2$.
- Hypoxia induced by early avian development facilitates correct avian development.
- Determination of the $O_2$ diffusion coefficient over eggshell and associated membranes.
- Increased $O_2$ consumption rates, a small gas cell, and early membrane properties facilitate the establishment of hypoxia within the porous eggshell.

## INTRODUCTION

Oxygen is fundamental for the viability of adult vertebrates. However, the perceived causality between sufficient $O_2$ and the existence of animal life overshadows a less intuitive relationship between low oxygen concentrations (hypoxia) and animal development and evolution.

Hypoxia promotes cell immaturity (stemness), which is key during cell migration, tissue formation, and tissue homeostasis (Simon and Keith, 2008). For example, mammalian blood and immune cells are continuously replenished from hematopoietic stem cells that reside in the hypoxic (<2%) bone marrow (Mantel et al., 2015); conditions that would be deemed *severely* hypoxic by marine biologists (Hofmann et al., 2011). In contrast, higher $O_2$ concentrations promote cell differentiation and less versatile cell fate spectra (Vaapil et al., 2012). These insights challenge the conventional view that high $O_2$ concentrations are permissive of the development and evolution of complex organisms, such as animals. Rather, a dualistic view on $O_2$ appears appropriate since mechanisms for harnessing hypoxia – in niches, phases, or through cellular capabilities – might have been beneficial traits during animal evolution (Hammarlund, 2019).

The cancer field has profoundly expanded our understanding of hypoxic cell signaling in mammalian tissues by focusing on the Hypoxia Inducible Factors (HIF). This family of transcription factors regulates hypoxic responses, such as angiogenesis, during both tumor and animal development and are noted in all bilateral animal phyla (Semenza, 2012; Mohlin et al., 2017; Hammarlund et al., 2018). HIF-α subunits are constitutively translated but only stabilized during hypoxia, to then lead to a response. This linkage is exemplified by the HIF stabilization in muscle cells during anaerobic workout, which induces the formation of new blood capillaries that subsequently enhance oxygenation of the tissue (Iyer et al., 1998). A complete hypoxic response therefore relies on the combination of environmental hypoxia and a cellular HIF response. As of today, these two components are often observed and discussed separately. Hypoxia and HIF are also viewed differently in different scientific fields. Contrasting views are held by the cancer field, where hypoxia represents stress, and the field of Earth history, where hypoxia represents an ancestral normalcy. Developmental biology provides an arena in the middle, in which studies can decipher how and when life orchestrates sensing and responses to fluctuations in $O_2$.

It remains generally unexplored when and how environmental hypoxia and HIF responses are established and interact during early animal development. Considering that a hypoxic environment is inferred to be important for proper mammalian development and that HIF responses are inferred to be critical for vertebrate organ development (Tian et al., 1998; Dunwoodie, 2009; Niklasson et al., 2020), avian development offers a conundrum by occurring within a porous, $O_2$-permeable membrane (the calcareous eggshell). The early embryo is an almost planar and two-dimensional (2D) piece of tissue (e.g., 5 cell layers thick at HH8, Hamburger and Hamilton, 1951), where most cells will be exposed to ambient $O_2$ concentrations within the egg. How, then, are its hypoxia-dependent processes first established? One such hypoxia-dependent process is the development of blood vessels. In theory, vascularization should be hampered by the eggshell being permeable to $O_2$. Still, as early as embryonic day (E) 2–3, the avian vascular system develops underneath the porous eggshell (Mortola, 2009).

Here, we hypothesize and explore the potential existence of an early phase of environmental hypoxia within chicken eggs and investigate whether such a phase, combined with HIF stabilization, facilitates correct development of the embryo. To address these questions, we determine $O_2$ transport over the eggshell through two technically independent methods. We also determine $O_2$ dynamics within the gas cell above the developing embryo (*in ovo*) and in the embryonic tissues (*ex ovo*) through microelectrode measurements. We note a phase of severely hypoxic conditions evolving in the first or second day of embryogenesis (E1-E2) and estimate how this phase is driven by increased $O_2$ consumption rates ($V_{O2}$). We further demonstrate the increase of HIF-α protein levels in the embryo at the phase of hypoxia and how incubation of eggs at normoxia (21%) over the first 4 days negatively affects avian embryogenesis.

# MATERIALS AND METHODS

## Direct Determination of $O_2$ During Early Embryogenesis, *in ovo* and in Tissue

Domestic Lohman Brown chick (*Gallus gallus*) eggs were delivered fresh to the laboratory (fertilized and unfertilized). $O_2$ concentrations within the gas cell of the eggs *(in ovo*) during the first 4 days of incubation and development were quantified using Clark type $O_2$ microelectrodes, with an internal reference and guard cathode maintaining low $O_2$ levels in the internal electrolyte (Revsbech, 1989). Microelectrodes with tips of 5–10 μm were utilized (Unisense Ox-10). The dimensions of fertilized eggs were measured, a small hole (∼2 mm) was drilled through the shell in the blunt end of the egg without rupturing the inner membrane, and the eggs were mounted with the blunt end down on a stand next to a micromanipulator with the microelectrode (**Figures 1A,B**). The hole was covered (Parafilm) to avoid leakage and enable an additional sensor calibration at the end of the experiment. The microelectrode was introduced into and upward through the egg to a position in the gas cell ∼1–2 mm below the upper eggshell surface. The gas cell is normally positioned at the blunt tip of the egg, but in our setup moved to the pointed tip (and the hole drilled in the blunt tip). To maintain 37.5°C around the egg during measurements, heating wires connected to a thermostat were placed on the stand and the whole setup was insulated with a Styrofoam box (**Supplementary Figure 1**). Microelectrode data were recorded using the SensorTrace Suite Software. Temperature (T), and relative humidity (RH) inside the incubator were recorded with a HOBO MX temp RH logger, and software. The microelectrode continuously recorded the change in $O_2$ concentrations within the egg's gas cell during incubation. To calibrate the electrode after the measurements, two holes (0.2 mm) were drilled through the eggshell at the pointy end of the egg, into which first (a) 100% air, and then (b) 100% $N_2$ was injected with a gas-tight glass syringe. The microelectrode readings during injections served as calibration points for (a) ∼21% and (b) 0% $O_2$. After opening the egg, the development of the embryo and the position of the tip of the glass electrode were noted. In parallel, microelectrodes were used to record $O_2$ concentrations within embryonal tissues *(ex ovo*), sampled from the first four days of incubation and development (**Figure 1C**). The oxygenation status of the embryonal tissue was determined using the same experimental setup as described in Niklasson et al. (2020). At each developmental stage between HH10 and HH24 as defined by Hamilton-Hamburger (HH) (Hamburger and Hamilton, 1951), embryos ($n \geq 3$) were extracted and $O_2$ was immediately measured in four positions. In each embryo, $O_2$ levels were determined in the sacral region (tail), the head, the heart, and the vagal region (back).

## HIF Protein Levels

Fertilized eggs were incubated for 2–5 days after which embryos were harvested for determination of protein expression. Upon opening the eggs, the developmental stage of embryos HH was determined using morphological features and the number of somites. Embryos and allantois were dissected and separated. The samples were subsequently pooled for different developmental stages (three replicates of 10–35 embryos for each sample). Samples were kept on ice, homogenized in a cooled tissue lyser (100 μl urea lysis buffer mixed with protease and phosphatase inhibitor cocktails) and stored at −80°C. Determination of protein expression was performed using western blotting. Cell lysates (70 μg) were separated by 10% SDS-PAGE (BioRad), transferred to nitrocellulose membrane, blocked with blocking buffer (BioRad), and imaged on a Biorad Chemidoc, using antibodies for HIF-1α (NB100-479 at dilution 1:500) and HIF-2α (ab199 at dilution 1:4000), normalized to b-actin (12004163).

## Exposing Early Avian Development to Normoxia

A hole (∼2 mm) was carefully drilled in the blunt end of fertilized eggs. The hole was drilled at an angle to prevent it from becoming covered by dried albumen during incubation, and shell membrane covering the holes was removed (**Supplementary Figure 2**). These eggs and control eggs were incubated in a humidity chamber to maintain full RH inside the eggs at 37.5°C for 2, 3, 4 and 5 days ($n = 20$–40 in each group and day, see **Supplementary Table 3**). At each endpoint, the developmental stage (HH; by morphology and number of somites) and the diameter of the surficial vascular system (allantois) were noted. Viable embryos were checked for possible developmental abnormalities. To simulate a 21% $O_2$ excess and test whether delayed development was caused by the drilled hole rather than the normoxia, some eggs ($n = 20$) were incubated unopened at 40% $O_2$ for 3 days. To test the effect of environmental hypoxia, eggs were also incubated for 1 to 3 days at 10% $O_2$ while opened ($n = 20$) and unopened ($n = 20$).

## Quantification of the $O_2$ Diffusion Coefficient of Eggshell

To fully understand $O_2$ dynamics within the egg, we determine the $O_2$ transport across the eggshell. $O_2$ diffusion across the eggshell is driven by differences in $O_2$ concentration on its in- and outside. Under quasi-steady state conditions, the diffusive $O_2$ flux ($F$) can be expressed by Fick's first law of diffusion (1).

$$F = -D_{O2}\frac{dC}{dz} \tag{1}$$

Where $F$ is the diffusive flux of $O_2$ (mol $m^{-2}$ $s^{-1}$), $D_{O2}$ the diffusion coefficient of $O_2$ in the eggshell material ($m^2$ $s^{-1}$), and $\frac{dC}{dz}$ the $O_2$ concentration gradient (mol $m^{-4}$) across the eggshell. Here, we assume that a majority of the resistance is in the eggshell proper. Eq. 1 can be approximated as Eq. 2.

$$F = -D_{O2}\frac{C - C_{amb}}{\Delta z} \tag{2}$$

Where $C$ is the $O_2$ concentration in the gas cell inside the egg, $C_{amb}$ the constant $O_2$ concentration imposed on the outside of the egg, and $\Delta z$ is thickness of the eggshell. Using this

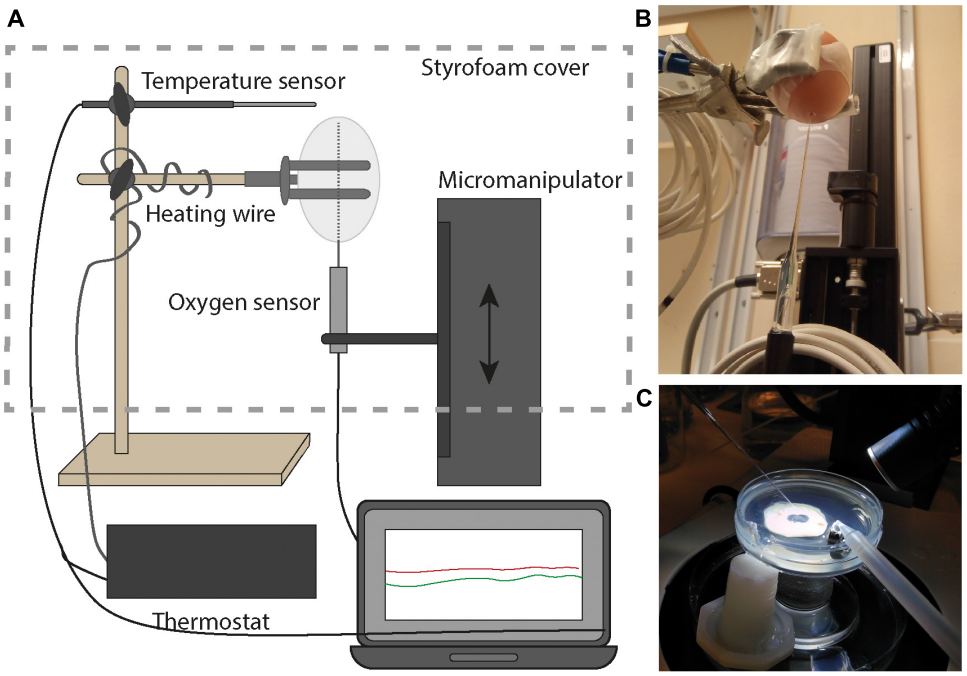


**FIGURE 1 |** Experimental setup for the direct quantification of $O_2$ concentrations in the egg and embryo. **(A)** During incubation, microelectrodes were logging measurements *in ovo* and temperature was maintained in a Styrofoam bell jar. **(B)** The microelectrode mounted on the micromanipulator entered the egg from below. **(C)** Microelectrodes determined $O_2$ concentrations within the embryo tissues *ex ovo* immediately upon opening, as described in Niklasson et al. (2020).

approximation in a mass balance equation for the gas cell inside the egg, we get Eq. 3.

$$V\frac{dC}{dt} = -AD_{O2}\frac{C - C_{amb}}{\Delta z} \quad (3)$$

Where $A$ ($m^2$) is the eggshell area around the gas cell and $V$ ($m^3$) is the volume of the gas cell. Eq. 3 has the analytical solution Eq. 4.

$$\frac{C - C_{amb}}{C_i - C_{amb}} = \exp\left(-\frac{AD_{O2}}{V\Delta z}t\right) \quad (4)$$

Where $C_i$ is the initial $O_2$ concentration in the gas cell ($t = 0$). From rearranging Eq. 4, we get Eq. 5.

$$C = C_{amb} + (C_i - C_{amb})\exp(-aD_{O2}t) \quad (5)$$

Where $a$ is a known constant equal to Eq. 6:

$$a = \frac{A}{\Delta z V} \quad (6)$$

$D_{O2}$ (the value sought) is a simple fitting constant that can be determined by least-square fitting of experimental data. We determined $D_{O2}$ based on empirical data from two independent approaches, one direct (using the microelectrodes) and one indirect (based on water vapor).

## Direct Quantification Using Microelectrodes *in ovo*

We used microelectrodes to determine $D_{O2}$ of the eggshell. Microelectrodes with tips of 5–10 μm were custom made and calibrated at the Hadal Center at University of Southern Denmark. The microelectrode was mounted on a motor-driven micromanipulator and introduced into and upward through the egg, as described above. The upper part of the egg was covered with a glass bell jar, sealed well below the gas cell (**Supplementary Figure 3**). The bell jar was connected to air or 100% $N_2$ that passed through two gas washers to maintain 100% RH (**Supplementary Figure 4**). The lower rim of the bell jar was sealed to the egg with a paste, through which a needle allowed gas escape. Microelectrode data were logged (Pyrofix software), as was temperature (HOBO MX temp RH logger). The setup settled for about 10 min with flow of air (∼21% $O_2$) while logging the microelectrode data. Subsequently, a switch was turned to quickly replenish the air with 100% $N_2$. The microelectrode continuously recorded the change in $O_2$ concentrations within the egg's gas cell. After reaching 0% $O_2$ in the gas cell, the switch was turned again, and the bell jar quickly re-filled with air. This was repeated 4–6 times per egg ($n = 9$). Calibration was performed as described above. After calibration, the top of the egg was opened, and height (max from eggshell at the pointy end to yolk) and diameter of the gas cell were measured with a caliper.

## Indirect Quantification via the Water Diffusion Coefficient for the Eggshell

To complement the direct microelectrode approach, we quantified the $H_2O$ diffusion coefficient ($D_{H2O}$) for the eggshells and converted this value to $D_{O2}$. Unfertilized eggs ($n = 59$) were weighed on arrival and incubated for 3 to 9 days at 37.5 ± 0.2°C and 22.8 ± 0.2% RH. During this time, they were weighed at the same time every day. At the end of the experiment the

width, heights and shell thickness of the eggs were measured using a caliper. The water evaporation rate over the entire eggshell area was assessed from the weight loss during the four days of incubation.

To calculate the diffusion coefficients, we made the following assumptions: (a) the weight change is constant between weight measurement points (interpolated linearly), (b) the diffusion coefficient is constant over time, (c) only water evaporates and across the entire eggshell area, (d) RH inside the egg is 100%, and (e) eggshell thickness is uniform. Assuming equal temperature inside and outside and 100% RH inside the egg, the water vapor concentration difference over the eggshell was calculated using the Arden Buck relation. To calculate the water vapor diffusion coefficient of eggshell, we re-applied (2). Assuming that the concentration gradient is in steady state, (2) can be rewritten as (7):

$$D_{H2O} = -\frac{\Delta N \Delta z}{\Delta C A \Delta t} \quad (7)$$

Where $\Delta N$ is water loss (moles), $\Delta z$ is eggshell thickness (m), $\Delta C$ is concentration difference of water vapor over the eggshell (mol $m^{-3}$) which is derived from RH, $A$ is the whole area over which water vapor can diffuse ($m^2$), and $\Delta t$ is time between measurements (s). $\Delta N$ can be calculated from the weight loss measured over time. The area of each egg $A$ was estimated by dividing its area into two ellipsoids, using its measured dimensions (**Supplementary Figure 4B**).

By measuring multiple eggs, an average value of the $D_{H2O}$ of eggshell could be determined, allowing the conversion to $D_{O2}$. At 310K and 1 atm, $D_{O2}$ ($O_2$ in air) = 0.2196 $cm^2$ $s^{-1}$ and $D_{O2}$ (water in air) = 0.2267 $cm^2$ $s^{-1}$, yielding a ratio of 0.9686 (Higgins and Binous, 2013).

## Determining $O_2$ Consumption

The chick embryo's oxygen consumption ($V_{O2}$) creates an $O_2$ pressure gradient, resulting in the flux of oxygen across the eggshell (Mortola, 2009). To assess the $O_2$ dynamics within the intact gas cell of the egg over time, we must combine the recording of $O_2$ concentrations with our assessments of the continuous influx of $O_2$ over the porous eggshell. We here ignore $O_2$ that is supplied to the gas cell from the egg white. Although $O_2$ is also transported from the egg white to the gas cell, we evaluate that the significantly slower diffusion of $O_2$ in liquid compared to gas allows us to ignore the $O_2$ contribution from the egg white. Thus, to determine the influx of molar $O_2$ over time, we assumed that $O_2$ only diffuses into the gas cell of the egg from the outside. The gas cell is located at the top of the egg in the setup used here. The transport into the egg gas cell is estimated using Eq. 8.

$$J = -\frac{D_{O2} A \Delta C}{\Delta z} \quad (8)$$

Where $J$ is diffusive transport (mol $s^{-1}$), $D_{O2}$ is the diffusion coefficient ($m^2$ $s^{-1}$), $A$ is the area of the gas-cell eggshell area ($m^2$), $\Delta C$ is the difference in $O_2$ concentration (mol $m^{-3}$), and $\Delta z$ is the eggshell thickness (m). The area increases over incubation, and to estimate this, we measured the increase of gas-cell volume inside the eggs over incubation. The volume of the gas cell was regularly measured by breaking the eggs open underwater, one side at a time, and funneling the air to an upside-down graduated cylinder filled with water. The evacuated gas was measured with that measuring flask. Overall, these estimates of the molar flow of $O_2$ over the eggshell, allow us to evaluate overall $V_{O2}$ during the first days of embryogenesis.

# RESULTS

## *In ovo* $O_2$ Measurements During Incubation Using Microelectrodes

Oxygen concentrations in the gas cell of unfertilized eggs were in equilibrium with air for up to 4 days in the incubator (**Supplementary Figure 5** and **Supplementary Table 1**). By contrast, fertilized eggs exhibited lower and variable $O_2$ concentrations in the gas cell during this time (**Figure 2A** and **Supplementary Table 2**). At E1 or E2, $O_2$ decreased to 10–50% of the starting levels. The distinct decrease in $O_2$ concentrations lasted for 6–24 h, after which $O_2$ levels increased but remained below the initial values until the end of the experiments (by E5 at the latest). All embryos had developed and were alive by the end of the experiment. Out of four eggs incubated over E3, three recorded a slight increase in gas-cell $O_2$ concentrations at E3.5.

Measurements of tissue oxygenation over the same time period demonstrated an overall decrease from near fully saturated at developmental stage HH10 (∼30 h or E1) to 20–30% of full saturation at HH 15 (∼E2), corresponding to atmospheric $O_2$ concentrations of 4–8% (**Figure 2B**). At developmental stage HH24 (∼E4), $O_2$ is <10% of full saturation, corresponding to atmospheric $O_2$ concentrations of 2% or less. The trend of tissue $O_2$ decreasing from nearly fully saturated to what corresponds to <2% of atmospheric $O_2$ concentrations is observed in the tail (sacral region), heart, back (vagal region), and head.

## HIF Protein Levels

HIF expression was investigated in both whole-embryo lysates and surrounding allantois. HIF-1α and HIF-2α expression increased between the HH10 and HH14 developmental time points in the embryos. HIF-1α expression was threefold and HIF-2α expression was fourfold higher in HH14 than HH10. Expression of HIF-1α was high in allantois at all stages, whereas HIF-2α was only expressed at HH10 (**Figure 2C**).

## Exposing Early Avian Development to Normoxia

To test the importance of the hypoxic phase, eggs were incubated with a drilled hole such that early development occurred under atmospheric $O_2$ (21%). Embryogenesis under

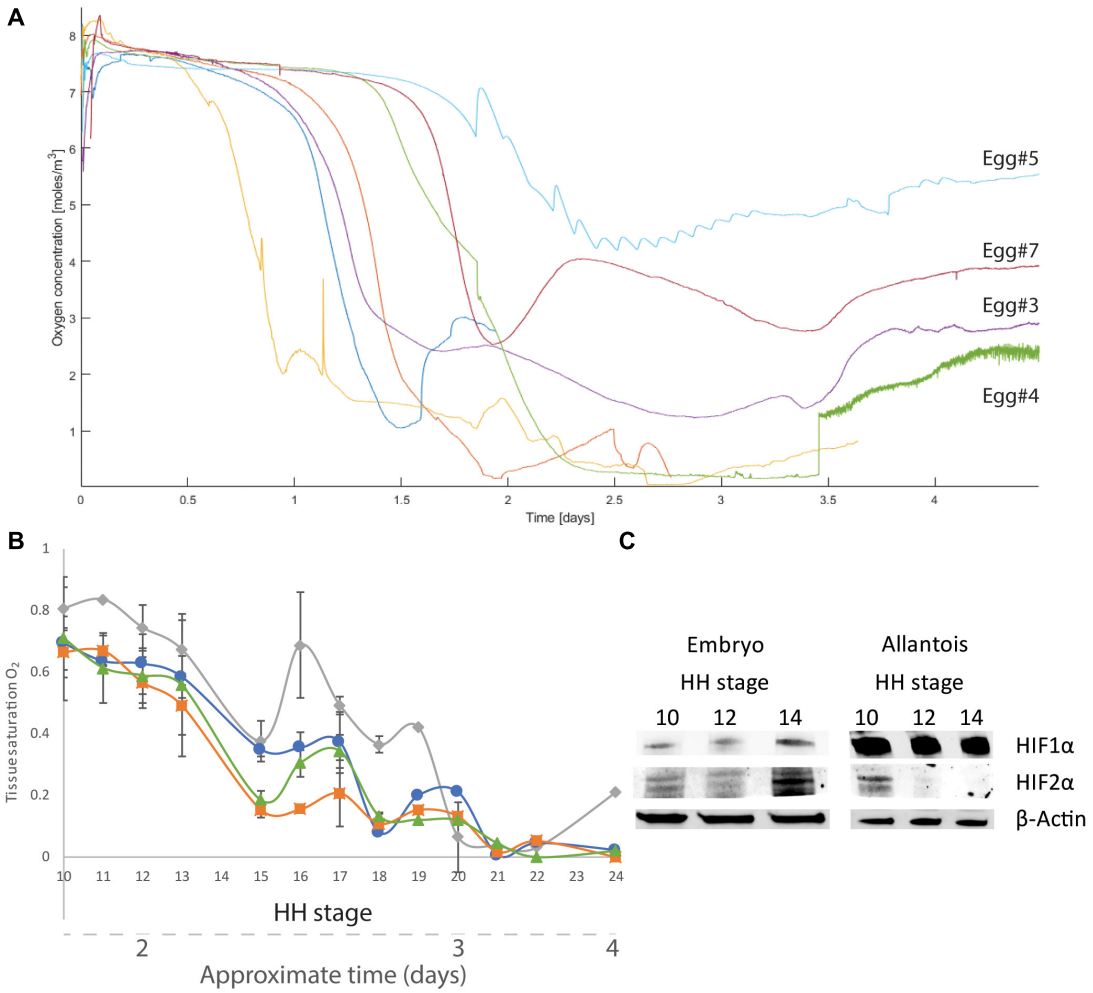


**FIGURE 2 |** $O_2$ concentrations near and in the embryo as well as HIF-α expression. **(A)** During incubation of fertilized eggs ($n = 7$), the gas cell saw a decrease in $O_2$ concentrations. **(B)** Tissue $O_2$ within the embryo measured *ex ovo* immediately after opening (full saturation under 21% atmospheric $O_2 = 1$). From HH10 (approximately E1) to HH24 (approximately E4), oxygenation in the sacral region (gray diamonds), head (blue circles), heart (green triangles), and vagal region (orange squares) demonstrate a decrease in $O_2$ (black bars represent standard error). **(C)** HIF expression based on whole-embryo samples. Expression of HIF-1α and HIF-2α is lower in samples from developmental stages HH10 and HH12 than in samples from stage HH14. In samples of the allantois, HIF-1α is strongly expressed from HH10 to HH14, while HIF-2α expression is detectable at HH10 but absent thereafter.

atmospheric $O_2$ for up to 5 days led to increased death rates of the embryos, compared to endogenous conditions. Average death rates for embryos incubated for 2–5 days were 7.5% for control and 59.8% for those with an opening (**Figure 3A**). Viable embryos in the opened eggs were significantly delayed in their development (**Figure 3B**) and their vascular system (allantois) was significantly smaller (**Figure 3C**) than that of embryos in the intact eggs (for statistical tests see **Supplementary Table 3**). The vascular systems of embryos in eggs with an opening to air (21% $O_2$) were morphologically deformed compared to those of control embryos (**Figure 3D** and **Supplementary Figures 6, 7**). Embryos incubated for 3 days at 40% $O_2$ in unopened eggs demonstrated a similar delay in development as for opened eggs, that is significant (**Supplementary Table 4**). Embryos in opened and unopened eggs that were incubated at 10% $O_2$ were at a similar developmental stage at E1 but all dead at E3 (**Supplementary Table 4**).

## Quantification of the $O_2$ Diffusion Coefficient of Eggshell

To understand the generation of the observed hypoxia, $O_2$ diffusion and flux over the eggshell was investigated. Continuous measurements of $O_2$ inside the gas cell after shifting ambient $O_2$ levels from 21 to 0% demonstrated that $O_2$ diffuses out of the gas cell over the eggshell within ∼20 min (**Figure 4A** and **Supplementary Table 5**). The eggshell thickness was on average 0.4 mm ($0.39{\cdot}10^{-3} \pm 0.04{\cdot}10^{-3}$ m) and its area on average 71 $cm^2$ ($7.1{\cdot}10^{-03} \pm 0.3{\cdot}10^{-03}$ $m^2$). Based on these data, $D_{O2}$ was determined as $3.37{\cdot}10^{-09}$ $0.14{*}10E^{-9}$ $m^2$ $s^{-1}$ (**Figure 4B**, crosses).

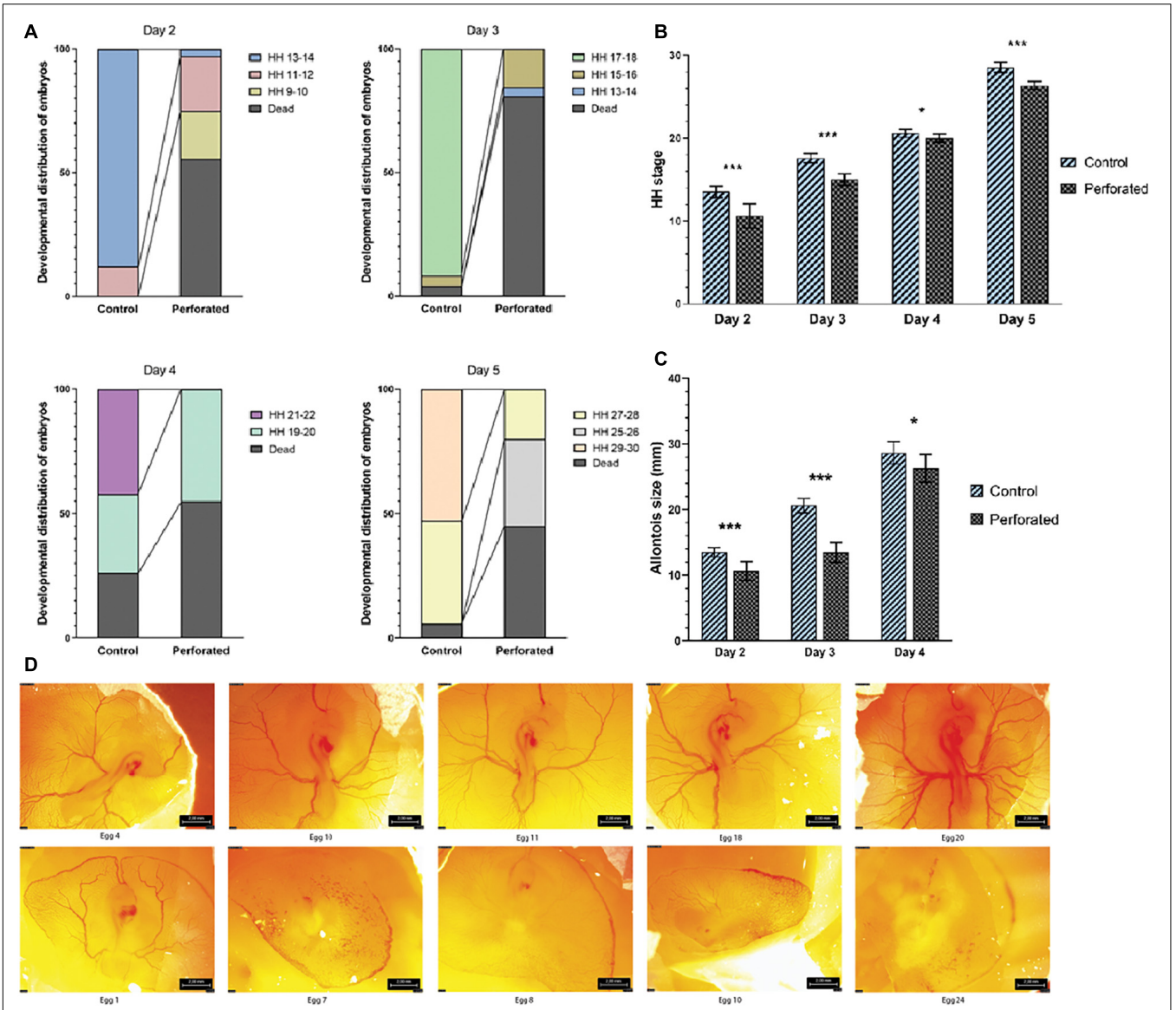


**FIGURE 3 |** Developmental effects of exposure to atmospheric $O_2$ during early embryogenesis. **(A)** The death rates (%) and developmental stage (HH) in treated (perforated) and control groups at day 2–4 of incubation. **(B)** The delay in developmental stage of living embryos is significant. **(C)** The surficial vascular system (allantois) diameter of embryos in the control group and the perforated group after 2 and 4 days of incubation. **(D)** Normal avian development after 3 days of incubation (upper) and after full access to atmospheric $O_2$ (lower). Scale bar: 2 mm. Error bars represent standard deviation. The asterisks denote a significant difference obtained through Mann-Whitney U tests for HH stages (discrete) and Welch's t-test for allantois size (continuous): $^{*}p \leq 0.05$; $^{***}p \leq 0.001$. For Day 2: $N_{control} = 33$, $N_{perforated} = 16$, $U = 34.5$. For Day 3: $N_{control} = 23$, $N_{perforated} = 5$, $U = 2$. For Day 4: $N_{control} = 14$, $N_{perforated} = 9$, $U = 27$. For Day 5: $N_{control} = 16$, $N_{perforated} = 11$, $U = 14$.

During the first 4 days, unfertilized eggs kept at 37.5°C lost on average 0.58 (±0.04) g $day^{-1}$ corresponding to a loss of ∼1.3% (**Supplementary Figure 8** and **Supplementary Tables 6, 7**). The corresponding water diffusion coefficient ($D_{H2O}$) was on average $10.27{\cdot}10^{-9} \pm 0.25{\cdot}10^{-9}$ $m^2$ $s^{-1}$. The value of $D_{O2}$ was determined as $9.95{\cdot}10^{-9} \pm 0.24{\cdot}10^{-9}$ $m^2$ $s^{-1}$ (**Figure 4B**, circles). The indirectly determined $D_{O2}$ values were largely constant for the first 4 days of incubation (**Figure 4C**).

## Determining $O_2$ Consumption

We used the indirectly determined $D_{O2}$, the gas-cell volume increase (**Supplementary Table 8**), and the direct measurements of $O_2$ concentrations within the gas cell of eggs that were viable for 4 days or more ($n = 4$) to determine $O_2$ consumption within the eggs. Using the indirectly determined $D_{O2}$ allows an estimation of flux of oxygen into the egg. When the hypoxic phase is generated inside the eggs, $V_{O2}$ increases 3–7 times, from

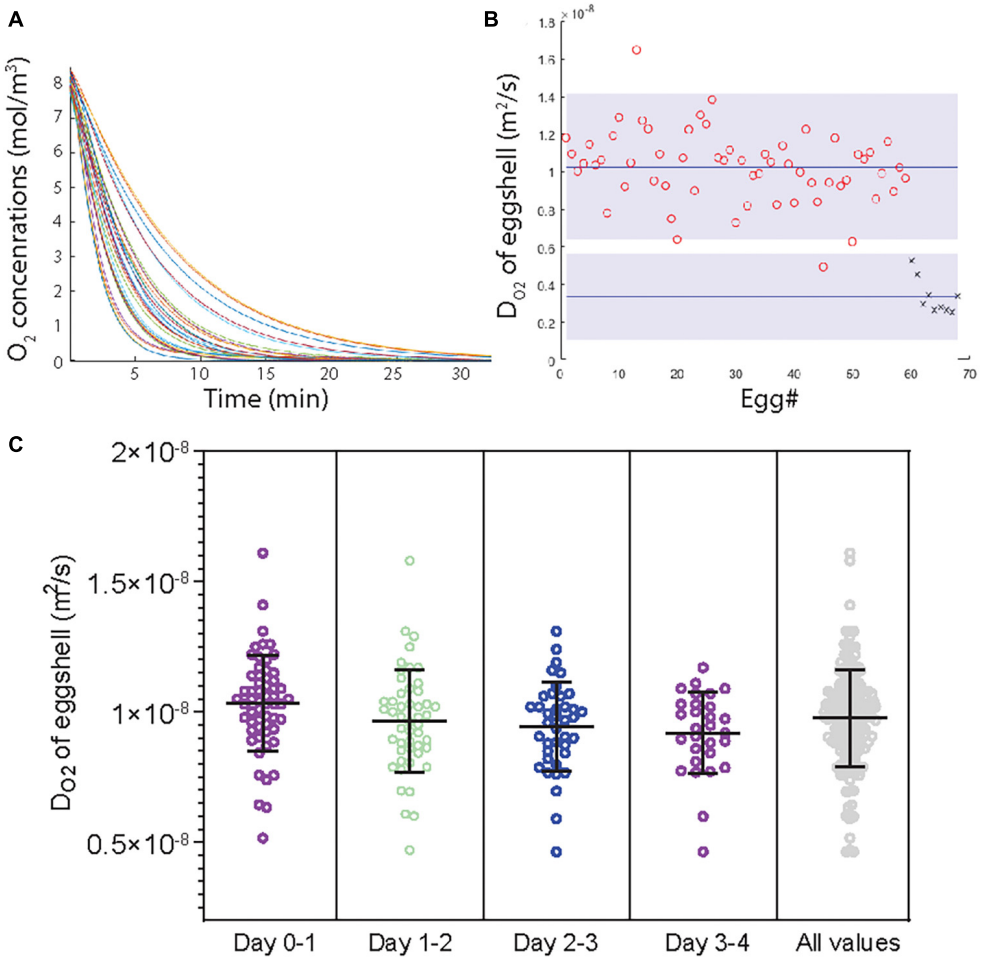


**FIGURE 4 |** Quantifications of $D_{O2}$. **(A)** The loss of $O_2$ from the gas cell of the egg when $N_2$ surrounds the egg, as measured with microelectrodes *in ovo*. **(B)** A comparison of $D_{O2}$ values obtained through direct (crosses; $n = 9$) and indirect (circles, $n = 59$) methods. The average is marked (blue line), as is the significance ($p < 0.05$) as obtained through the Welch *t*-test (gray field). **(C)** The indirectly determined $D_{O2}$ (based on water evaporation over four days) is also reported for each day (1–4) and overall ($n = 59$). Horizontal lines denote the mean value for each time interval and the error bars represent the standard deviation from the mean.

$\sim 2{\cdot}10^{-8}$ to $6{-}14{\cdot}10^{-8}$ mol $s^{-1}$ (**Figure 5**). Flux was also estimated using the directly determined $D_{O2}$ values (**Supplementary Figure 10**).

## DISCUSSION

We observed a distinct phase of hypoxia during early chick embryogenesis, despite the eggshell being a membrane permeable to $O_2$. The phase of hypoxia associates with HIF-α stabilization and appears to be important for normal development of the embryo and the vascular system. The fact that the eggshell is permeable, combined with the observed low $O_2$ concentrations within the gas cell, lead us to infer that the hypoxic phase must have resulted from increased $O_2$ consumption rates ($V_{O2}$). Below, we discuss the estimates of $O_2$ diffusion, the presence and role of a hypoxic phase during early avian development, and the broader implications of our findings.

### A Distinct and Critical Phase of Environmental Hypoxia During Avian Embryogenesis

Hypoxia developed in the gas cell during E2-E4, after which $O_2$ levels increased but remained below air saturation (**Figure 2A**). To our knowledge, the presence of hypoxia in the uppermost liquid of eggs (through embryo, allantois, albumen or yolk) has been measured only once before, at time points during E0-E4 (Lomholt, 1984). After removing the eggshell and outer membrane, these measurements were discrete and blindly aiming through the inner membrane to unknown positions in the liquid. $O_2$ levels were found to decrease when hitting the liquid (and possibly the embryo or allantois), down to 10% of initial levels (Lomholt, 1984). In the tissues of similarly early embryos in our study, oxygen levels follow on the observed phase of hypoxia (**Figure 2B**). The decrease is notable in vagal and sacral regions, as well as in the heart and head, and is similar to an earlier observation that $O_2$ levels in the trunk region decrease within

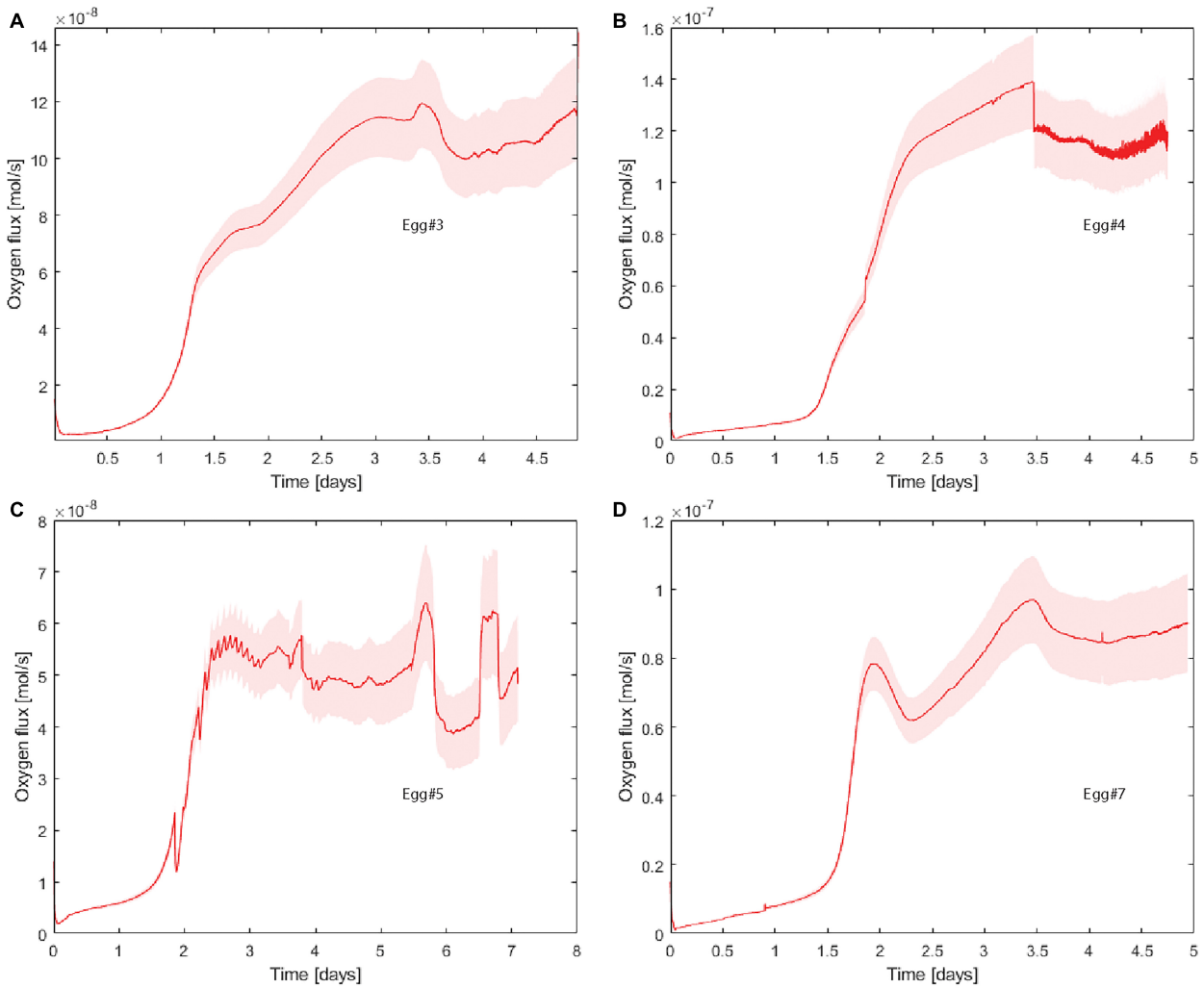


**FIGURE 5 |** Influx of $O_2$ (mol $s^{-1}$) during incubation of Eggs #3 **(A)**, #4 **(B)**, #5 **(C)**, and #7 **(D)**. These estimates were based on our indirectly determined $D_{O2}$ value. The surrounding field (pink) reflects error propagation for our uncertainties (D, A, z, and V).

the same time interval (Niklasson et al., 2020). Previously, $O_2$ levels in both tissue and blood have been measured at E4-E6 and noted to be at 20–30% of full saturation (Meuer et al., 1992). Therefore, our observations of hypoxia in the gas cell and embryonal tissues are consistent with previous observations and add resolution to the first four days of embryogenesis. In the following, we will discuss to what extent the phase of hypoxia matters for embryo development.

Developmental challenges related to hypoxia have been thoroughly investigated, but the focus has generally been on the later stages of development. For example, eggs exposed to hypoxia at E15-E18 (hatching occurs at E21) result in chicks with decreased hatch size (e.g., Visschedijk et al., 1980; Metcalfe et al., 1981). By contrast, observations from early development indicate a different role of hypoxia. Firstly, embryos at E1-E2 have been noted to 'tolerate' hypoxia better (measured through survival after exposure to decreasing $O_2$ levels) than those at E3-E4, which has led to the suggestion that the early embryo utilizes anaerobic metabolism (Grabowski and Paar, 1958; Kučera et al., 1984). This would be consistent with observations that mitochondria cristae remain poorly developed at ∼E1.5 (Scully et al., 2016) and agrees with the timing of the hypoxic phase that we observe here. Secondly, the nascent vascular systems in chick embryos younger than ∼E3 and zebra fish embryos younger than ∼E4 do not primarily provide $O_2$ transport, since blocking their hemoglobin (with CO) does not affect development (Cirotto and Arangi, 1989; Pelster and Burggren, 1996). Thirdly, hypoxia and HIF-α are associated with the correct development of organs and vascular systems in e.g., chicken, quail, xenopus, and zebra fish (Naňka et al., 2006; Ota et al., 2007; Barriga et al., 2013; Scully et al., 2016; Niklasson et al., 2020). For example, measurements of $O_2$ levels within early zebra fish embryos (∼26 h after fertilization), right before vascularization, demonstrate hypoxic conditions (<2%) (Kranenbarg et al., 2003), or *severely* hypoxic, depending on context. By contrast, development in eggs under hyperoxia

(100%) leads to an underdeveloped chick embryo vascular system (Höper and Jahn, 1995), similar to what we also note when eggs are opened to normoxia (21%) (**Figure 3**). That both 21% and 100% $O_2$, in this regard, have similar effects on development suggests that both indeed are hyperoxic compared to a phase of critical hypoxia. Fourthly, the necessity of hypoxia (in the tissue of quail embryos) appears to precede the expression of *HIF1A* and angiogenesis (Naňka et al., 2006). We note that hypoxia in both the egg gas cell and chick embryo tissue also precedes HIF-α expression in the embryos. Expression of both HIF-1α and HIF-2α proteins increased after the onset of the distinct hypoxic phase (**Figure 2C**). During the phase of hypoxia, the expression of particularly HIF-1α shifts from being predominantly expressed in the allantois (HH10-HH12) to being predominantly expressed within the tissues (HH14). Previously, HIF-1α has been observed to be expressed primarily in the allantois at E0-E2 and within the embryo tissues during later stages (E4-E6) (Meuer et al., 1992; Ota et al., 2007). Our data therefore complement and corroborate previous studies observing that hypoxia determines the stabilization of HIF-1α in particular, and directs proper chick embryo development in general.

In summary, our results align with earlier work finding that both hypoxia and HIF-α responses are prerequisites for proper development. We furthermore propose that the phase of environmental hypoxia is a prerequisite to kick-start the genetically determined cellular hypoxia-response machineries. Without the initial phase of hypoxia, avian embryos might risk dying from lack of 'lack of oxygen'.

## Increased $O_2$ Consumption Rates ($V_{O2}$) and Properties of the Eggshell Generate Hypoxia

The phase of hypoxia that we observed in the gas cell is not induced by hypoxia in the external environment. Air and $O_2$ continuously permeate through the porous eggshell. This means that transient hypoxia must be coupled to increased $V_{O2}$ within the embryo itself. The lower the gas-cell $O_2$ that we measured at E2-3, the higher the embryo's $V_{O2}$. That $O_2$ is necessary is indicated by our incubations at 10% $O_2$, where no embryos survive from E1 to E3. That also the biologically induced phase of hypoxia is important is indicated by incubations at hyperoxia (at 21% $O_2$ with perforated eggshells or at 40% $O_2$ with intact eggshells) where death rates are higher and development delayed (most pronounced around E3). To determine how this critical phase of hypoxia is generated, we investigated oxygen diffusion across the eggshell.

The oxygen diffusion coefficient ($D_{O2}$) of eggshell (and its outer and inner membranes) constitutes a necessary component to calculate the rate by which $O_2$ diffuses into the egg during the phase of hypoxia. Our $D_{O2}$ values obtained through microelectrodes (directly *in ovo*) are ∼30% lower than those obtained through water evaporation (indirectly via water vapor) (**Figure 4B**). This discrepancy could be due to several factors and we start by comparing our values to those reported in previous work. To our knowledge, only two previous investigations using microelectrodes have aimed to directly determine $O_2$ flux across the eggshell. Most other efforts have focused on the evaluation of the conductivity of water, which can be converted to $D_{O2}$ values (see **Table 1**, also for references and notes on our conversions).

The directly determined values of $D_{O2}$ range from $0.03{\cdot}10^{-8}$ to $1.11{\cdot}10^{-8}$ $m^2$ $s^{-1}$, our value being in the middle (Wangensteen et al., 1970/71; Kayar et al., 1981). Wangensteen et al. (1970/71) report the highest $D_{O2}$ value (see SI for conversions), which differs in that it represents flux through the eggshell only. In the given study, a cap of the eggshell was removed, cleaned of its inner and outer membranes, and mounted on a board where gases could be altered and measured on the inside of the cap (Wangensteen et al., 1970/71). Since high $D_{O2}$ values represent higher permeability, this value can be ascribed to the lack of membranes. Kayar et al. (1981) report the lowest $D_{O2}$ value, which was also obtained by measuring diffusion across a removed cap of eggshell, fluxing the inside with $N_2$ and then measuring the rate by which $O_2$ concentrations increased on the inside. However, the given study differs from others by (i) how eggs were incubated at 37°C until manipulation and measurements, and (ii) how the inner and outer membranes were removed sequentially to discriminate their and the eggshell's respective values of $D_{O2}$ over time. This study concludes that the permeability of the inner membrane increases 10-fold at E4-E6 (Kayar et al., 1981). That inner membrane properties change after the first days of development is supported by how, in quail eggs, the membrane thins from 74 nm at E2 to 35 nm at E10 (Yoshizaki and Saito, 2002). Our directly determined $D_{O2}$ value is 10 times higher than those determined by Kayar et al. (1981) at E3-E4 and similar to the value they obtained at E7. Part of the difference could be ascribed to a technical challenge in our setup regarding how to precisely determine the geometrical parameters of the gas cell. After drilling holes into the gas cell and calibrating the microelectrode, egg white was prone to exit the egg along the microelectrode. This loss of egg white may have led to an overestimation of the gas-cell volume and the eggshell area covering it, as well as to a lower $D_{O2}$ value. However, even if we adjust the gas-cell volume to 1/3 of the measured value (and adjust gas-cell area correspondingly), the resulting manipulated $D_{O2}$ value ($\sim 0.15{\cdot}10^{-8}$ $m^2$ $s^{-1}$, **Supplementary Figure 9**) is still higher than the $D_{O2}$ for E3-E4 reported in the study by Kayar et al. (1981). Therefore, it could also be possible that the temperature or the developing embryo influences the properties of the inner membrane during early embryogenesis, and that this influence is reflected in the uniquely low E3-E4 $D_{O2}$ values of Kayar et al. (1981). Due to the uncertainty as to why these directly determined $D_{O2}$ values differ, we also determined $D_{O2}$ indirectly via water vapor.

Most efforts to determine the value of $D_{O2}$ have been indirect and based on estimated water flux across the eggshell. In these, weight loss over time combined with differences in water vapor pressure over the eggshell is used to calculate water conductivity ($G$), expressed as e.g., $mg \times day^{-1} \times torr^{-1}$ (Wangensteen and Rahn, 1970; Ar et al., 1974; Paganelli et al., 1975; Rahn et al., 1975; Ar and Rahn, 1985; Seymour and Visschedijk, 1988; Wagner-Amos and Seymour, 2002). To compare available data, we assumed values for the eggshell area and thickness in

**TABLE 1 |** Comparison of water and oxygen diffusion coefficients of chicken eggshell.

| Publication | Indirect/direct | $D_{H2O}$ ($m^2/s$)× $10^{-8}$ | $D_{O2}$ ($m^2/s$)× $10^{-8}$ | Shell area ($cm^2$) | Temp (°C) at exp. | Note |
|---|---|---|---|---|---|---|
| **This study** | **Direct** | | **0.34 ± 0.01** | | **22** | **With membranes** |
| Wangensteen et al., 1970/71 | Direct (*K*)* | | 0.83–1.11 | | 25 | No membranes |
| Kayar et al., 1981 | Direct (*K*)* | | 0.03–0.04 | | 37 | E3-E4 |
| | | | 0.23–0.31 | | 37 | E7 |
| **This study** | **Indirect** | **1.23 ± 0.2** | **0.99 ± 0.24** | **71** | **37.5** | **With membranes** |
| Paganelli et al., 1975 | Indirect (*G*)** | 0.58–0.77 | 0.56–0.74 | 66.4 | 24.5 | Avg A for all eggs |
| Seymour and Visschedijk, 1988 | Indirect (*G*)** | 0.67–0.89 | 0.65–0.86 | 68*** | 25 | |
| Ar et al., 1974 | Indirect (*G*)** | 0.76–1.01 | 0.73–0.98 | 68*** | 20–25 | |
| Rahn and Paganelli, 1990 | Indirect (*G*)** | 0.11–0.14 | 0.10–0.14 | 68*** | | Several bird species |

*The directly determined $D_{O2}$ values are first converted (marked *) from oxygen permeability measurements (K) to water conductivity (G) and then into $D_{O2}$. The indirectly determined $D_{O2}$ values are converted (marked **) from water conductivity measurements (G). The conversions have been made using the eggshell area reported in the given publication or a "standard" area of 68 $cm^2$ (marked ***). For the conversion of G to $D_{O2}$, eggshell thickness (z) of 0.3 mm and also 0.4 mm are used, which leads to the range in those $D_{O2}$ values. Wangensteen et al. used their diffusive permeability of the eggshell (K; expressed as $cm^3$ STP × $sec^{-1}$ × $cm^{-2}$ × mm $Hg^{-1}$) to describe diffusion of both $O_2$ and water vapor (Wangensteen and Rahn, 1970 and Wangensteen et al., 1970/71). In a follow-up study, water vapor conductivity (G) divided by the total area of the egg is reported to equal the K value (Paganelli et al., 1975), which allows us to compare their value (K') to our $D_{O2}$. The conversion from G to $D_{O2}$ is made by using the data available in each publication. G is multiplied by the partial pressure difference and then converted to moles/day. The partial pressure difference, using the ideal gas law, is converted into moles/$cm^3$. These two can then be used in Eq. 6 to calculate a $D_{O2}$ value. Since most of these publications lack data on total eggshell area and eggshell thickness, assumptions have been used for these parameters. For the directly determined $D_{O2}$ values, the area cancels itself out and is therefore left out of the table.*

previous studies (if not stated in the respective publications). Additionally, we converted reported values of $D_{H2O}$ to $D_{O2}$ to be able to directly compare to our obtained value (**Table 1**). Although experimental setups differ in terms of temperature and avian species, our indirectly obtained $D_{O2}$ value is largely consistent with those determined previously based on water evaporation. While the general consistency of the indirectly determined $D_{O2}$ values is encouraging, the discrepancy in the $D_{O2}$ values determined directly (by recording $O_2$ flux) and indirectly (based on water evaporation) lingers. It is impossible that this reflects a real difference in the membrane properties, and that water and $O_2$ are being transported through the membrane at different rates during early embryogenesis. The properties of the inner membrane may change through incubation or embryogenesis, and it is known that the permeability of the membrane with respect to $O_2$ changes around E4 (Kutchai and Steen, 1971; Kayar et al., 1981). Thus, our initial assumption that the majority of resistance is in the eggshell proper does not appear to hold. The possibly different and changing diffusive properties of eggshell versus membranes require further studies. Although beyond the scope of this paper, the indication that egg membrane properties may play a discriminatory role for transporting gases ($O_2$ and $H_2O$) at different rates later in embryo development is noteworthy. Nevertheless, because of the consistency between our indirectly determined $D_{O2}$ value and those reported by others, we use this value when calculating the influx of $O_2$ during early embryogenesis and the hypoxic phase.

Defining the $D_{O2}$ of eggshell allowed us to estimate the theoretical maximum influx of $O_2$ per time unit ($F$) at the beginning and end of incubation. At day zero, the eggshell area covering the gas cell (normally at the blunt end of the egg) constitutes ∼5% of the total eggshell area ($3.8{\cdot}10^{-04}$ $m^2$). At E20, the chick is fully developed and $O_2$ can be transported across the entire eggshell area ($7.1{\cdot}10^{-03}$ $m^2$). Based on calculation (8) and our indirectly determined value of $D_{O2}$, the flux of $O_2$ can, theoretically, increase from $0.8{\cdot}10^{-07}$ mol $s^{-1}$ at E1 to $15{\cdot}10^{-07}$ mol $s^{-1}$ at E20. This theoretical 20-fold increase of flux can be compared to empirical data (via e.g., respirometry) reporting a fivefold increase in chick-embryo $V_{O2}$ between E1 ($0.7{\cdot}10^{-07}$ mol $s^{-1}$) and E20 ($3.7{\cdot}10^{-07}$ mol $s^{-1}$) just before hatching (Bartels and Baumann, 1972; Mortola, 2009 and references herein). This would support previous observations that, rather than limited $O_2$ supply, late stages of development are sensitive to the build-up of $CO_2$ that also initiates hatching (Tazawa et al., 1988; Mortola, 2009). Here, however, the early and dramatic increase in the chick-embryo $V_{O2}$ is of particular importance in that it may be facilitating the observed phase of gas-cell hypoxia.

Logging $O_2$ concentrations within the gas cell and estimating the continuous influx of $O_2$ over the porous eggshell allow us to visualize the dramatic increase in $V_{O2}$ that induces the hypoxic phase at E2 (**Figure 5**). The magnitude of the increase in $V_{O2}$ estimated in the present study, based on $O_2$ data from the gas cell, is largely consistent with the $V_{O2}$ increase observed previously based on respirometry data of the early chick embryos (Bartels and Baumann, 1972). Respirometry data reflect a doubling of $V_{O2}$ between HH4 or ∼E0.75 ($0.7{\cdot}10^{-07}$ mol $s^{-1}$) and HH8 or ∼E1.25 ($1.4{\cdot}10^{-07}$ mol $s^{-1}$). Although increased $V_{O2}$ is detected in the gas cell at ∼E1, the most dramatic increase occurs slightly later. $V_{O2}$ roughly doubles from about $0.6{\cdot}10^{-07}$ mol $s^{-1}$ at E1-E2 to $1.4{\cdot}10^{-07}$ mol $s^{-1}$ at E2-E3 (**Figure 5**). That an increase in chick-embryo $V_{O2}$ precedes the gas-cell $O_2$ dynamics reported here, and both being of a corresponding magnitude, makes us infer that increased cell proliferation in the growing embryo is indeed inducing the observed hypoxic phase. This conclusion is supported by the fact that a fourfold increase in protein synthesis is known to occur between HH4 and HH8 and that neither egg yolk nor white was found to bind $O_2$ to a higher

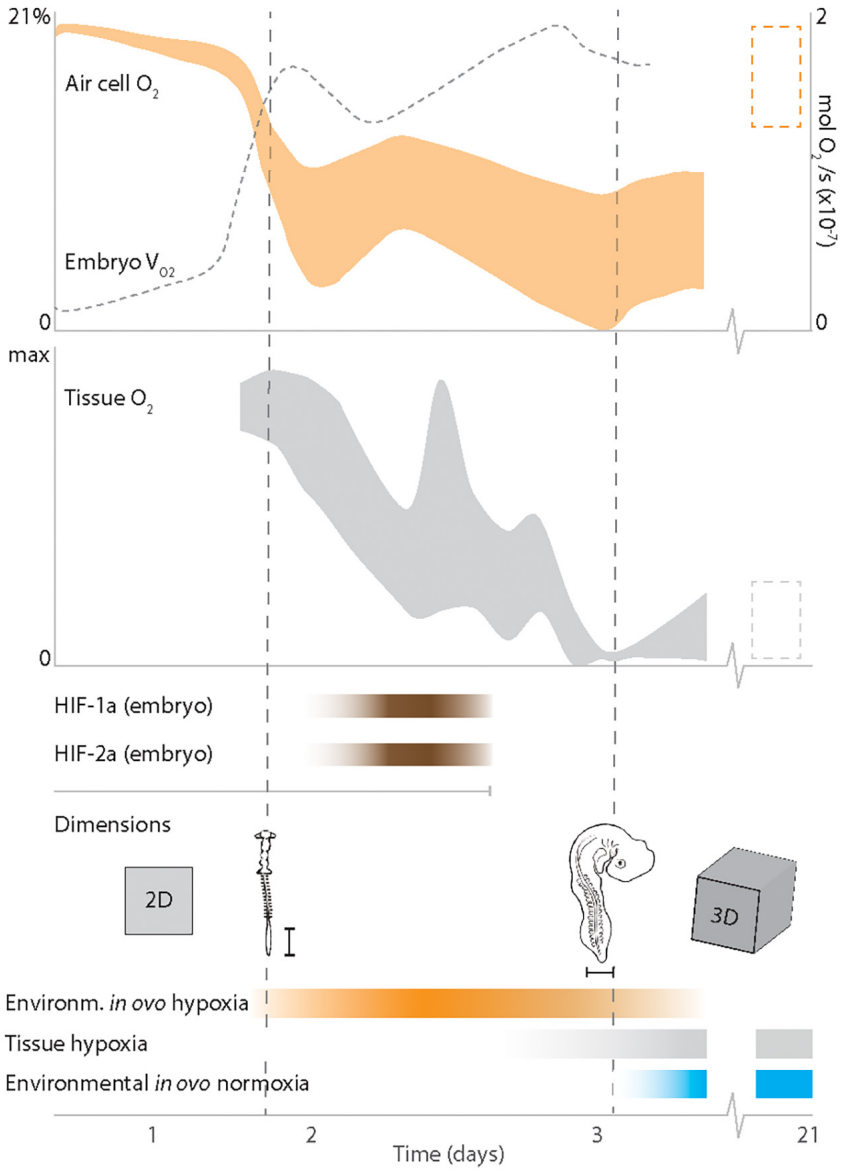


**FIGURE 6 |** Our interpretation of data presented here and in previous work. Increased oxygen consumption rates within the embryo ($V_{O2}$) together with physical parameters of the eggshell and gas cell generate a distinct phase of hypoxia in the gas cell and tissues within the first two days of avian development. The presumed $O_2$ status at the end of embryogenesis (E21) is depicted with a box (dashed) that spans over (in the gas cell) normoxic and (in tissue) hypoxic (or physiologically normoxic) conditions. Environmental hypoxia assists the embryo in growing from the 2D shape and enables HIF-driven angiogenesis. The phase of hypoxia-dependent development continues until hypoxia and hypoxia-responses are regulated internally within the embryo's 3D-shape, and the phase of normoxia-dependent development ensues. The dimension of the embryo at HH10 and HH18 are approximate. Scale bar is 1 mm.

degree during incubation (Kučera et al., 1984). Based on our measurements, hypoxia becomes less pronounced at E3.5 in three out of four experiments and while the onset and degree of hypoxia vary between eggs, the timepoint at which hypoxia lessens does not (**Figure 2A**). We ascribe the observation that the degree of hypoxia seems to consistently lessen at E3.5 to the previously described change in the properties of the inner membrane at this stage (Kayar et al., 1981; Yoshizaki and Saito, 2002). Thus, membrane properties and gas-cell dimensions, as well as variations in the $O_2$ consumption rates during early incubation, appear to be coupled to the induction of the hypoxic phase.

## Hypoxia as a Requirement for Early Vertebrate Development

We report a distinct phase of hypoxia during early avian development that we believe to be generated by a combination of eggshell properties and variations in the embryo's $V_{O2}$. This hypoxic phase appears to be critical for normal chick development.

These findings are relevant since they challenge the thinking on the importance of oxygen. In previous studies, it is commonly explored how the embryo is able to access *enough* oxygen through the eggshell (e.g., Kayar et al., 1981). Indeed, all the while, avian embryogenesis within a porous eggshell posed a conundrum for the exact opposite reason – how to generate sufficiently low $O_2$ concentrations. These findings may also suggest that the timing of the hypoxic phase occurs at a critical transition when the embryo evolves from a planar fetus shape to a 3D form. After this transition, the embryo's internal cells and chemistry become more sheltered from the immediate impact of the chemistry in the surrounding environment. We suggest that this transition reflects a divide between the first phase of embryogenesis, which is coupled to hypoxia dependency, and a second phase that is somewhat de-coupled from environmental hypoxia (**Figure 6**).

The latter phase of vertebrate development, which requires access to normoxia, accords well with the well-understood relationship between oxygen and animal development (e.g., Künzel et al., 1992). This phase likely comprises most of the development. For example, chicken hatch size has been observed to increase when incubation takes place under hyperoxic conditions (100% $O_2$) – if these are imposed after E3 (Cruz and Romanoff, 1944). This would suggest that the phase of hypoxia observed by us plays its role for the time we observed it (up until E3), after which an excess of $O_2$ can become beneficial for growth. So far, investigations into how vertebrate development requires certain levels of $O_2$ have overshadowed those into hypoxia-dependency. However, many studies have also pointed toward a necessity for an association between environmental hypoxia and the genetically determined hypoxic responses. The necessity of either hypoxia or HIF-α for development is demonstrated during the early stages of bird, fish, reptile, and mammal development (Grabowski and Paar, 1958; Lomholt, 1984; Meuer and Baumann, 1988; Kranenbarg et al., 2003; Niklasson et al., 2020). A full review of the complex impacts of hypoxia during development is beyond the scope of this paper. Here, however, we suggest it plausible that if the combined properties of the membrane and growth rates during avian development generate hypoxia, similar dynamics to govern hypoxia may be operating during the development of other vertebrates as well.

### Hypoxia Connects Animal and Tumor Evolution

That hypoxia would be present broadly during animal development resonates with how animals diversified on Earth under an atmosphere that was likely low enough in $O_2$ to be defined as "hypoxic" by modern standards (Hammarlund et al., 2018). The generation of hypoxia during development also resonates with how hypoxia is necessary to the function of cellular mechanisms that sense fluctuations in oxygen. The success of those eukaryotic clades that diversified on Earth's surface – animals, plants and fungi – has been associated with functionally similar cellular mechanisms for sensing oxygen fluctuations (Hammarlund et al., 2020). Considering this framework, it appears less surprising that cancer cells can use both hypoxia and HIF-mechanisms to their advantage. Indeed, to view hypoxia as a necessity and ancestral norm can challenge how we interrogate the varying roles of oxygen over tumor evolution. While a long-standing focus has been to explore how cancer cells cope with tumor hypoxia, it may be a larger enigma how circulating cancer cells can cope with oxygenated conditions in the blood stream. Future work should further elucidate how, when, and to what extent hypoxia is essential for animal development and health.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

According to Swedish regulations (Jordbruksverkets föreskrift L150, §5) work on chick embryos younger than embryonic day 13 do not require Institutional Animal Care and Use Committee oversight.

## AUTHOR CONTRIBUTIONS

EUH, SM, PB, and RNG designed the experiments. CC, EUH, PFN, and NE performed the experiments. PFN, NE, PB, RNG, and EUH evaluated physical parameters and statistics. CC, PFN, NE, SM, ERH, PB, RNG, and EUH wrote the manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

EUH received funding from the ParadOX-ERC Starting Grant (No. 96948) and from the Swedish Research Council (Grant 2019-05254). PB received funding from the National Science Foundation (United States; Grants: OCE-1851424 and OCE-1824144). RNG received funding from HADES-ERC Advanced Grant (No. 669947) and the Danish National Research Foundation through the Danish Center for Hadal Research, HADAL (No. DNRF145).

## ACKNOWLEDGMENTS

We are very grateful to Sara Egemose, Heidi G. Jensen, and Anni Glud for sharing technical expertise and scientific enthusiasm while developing the project. We thank Arne Johansson at Medicon Village for ample supply of eggs and valuable discussions. We also very much appreciate the reliable support from Elinn Johansson, Lucas Ahlm, and Elina Fredlund.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.675800/full#supplementary-material

## REFERENCES


Ar, A., Paganelli, C. V., Reeves, R. B., Greene, D. G., and Rahn, H. (1974). The Avian Egg: Water Vapor Conductance, Shell Thickness, and Functional Pore Area. *Condor* 76, 153–158. doi: 10.2307/1366725

Ar, A., and Rahn, H. (1985). Pores in avian eggshells: Gas conductance, gas exchange and embryonic growth rate. *Respir. Physiol.* 61, 1–20. doi: 10.1016/0034-5687(85)90024-6

Barriga, E. H., Maxwell, P. H., Reyes, A. E., and Mayor, R. (2013). The hypoxia factor Hif-1α controls neural crest chemotaxis and epithelial to mesenchymal transition. *J. Cell Biol.* 201, 759–776. doi: 10.1083/jcb.201212100

Bartels, H., and Baumann, F. (1972). Metabolic rate of early embryos (4–22 somites) at varying oxygen pressures. *Respir. Physiol.* 16, 1–15. doi: 10.1016/0034-5687(72)90083-7

Cirotto, C., and Arangi, I. (1989). Chick embryo survival under acute carbon monoxide challenges. *Comparat. Biochem. Physiol. Part A* 94, 117–123. doi: 10.1016/0300-9629(89)90794-9

Cruz, S. R., and Romanoff, A. L. (1944). Effect of Oxygen Concentration on the Development of the Chick Embryo. *Physiol. Zool.* 17, 184–187. doi: 10.1086/physzool.17.2.30151721

Dunwoodie, S. L. (2009). The Role of Hypoxia in Development of the Mammalian Embryo. *Devel. Cell* 17, 755–773. doi: 10.1016/j.devcel.2009.11.008

Grabowski, C. T., and Paar, J. A. (1958). The teratogenic effects of graded doses of hypoxia on the chick embryo. *Am. J. Anat.* 103, 313–347. doi: 10.1002/aja.1001030302

Hamburger, V., and Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Morphol.* 88, 49–92. doi: 10.1002/jmor.1050880104

Hammarlund, E. (2019). Harnessing hypoxia as an evolutionary driver for complex life. *Interf. Focus* 10:20190101. doi: 10.1098/rsfs.2019.0101

Hammarlund, E., Stedingk, K., and Påhlman, S. (2018). Refined control of cell stemness allowed animal evolution in the oxic realm. *Nat. Ecol. Evol.* 2, 220–228. doi: 10.1038/s41559-017-0410-5

Hammarlund, E. U., Flashman, E., Mohlin, S., and Licausi, F. (2020). Oxygen-sensing mechanisms across eukaryotic kingdoms and their roles in complex multicellularity. *Science* 370:eaba3512. doi: 10.1126/science.aba3512

Higgins, B. G., and Binous, H. (2013). *Binary Diffusion Coefficients for Gases in Wolfram Demonstrations Project*. URL: http://demonstrations.wolfram.com/BinaryDiffusionCoefficientsForGases/

Hofmann, A., Peltzer, E., Walz, P., and Brewer, P. (2011). Hypoxia by degrees: Establishing definitions for a changing ocean. *Oceanogr. Res. Papers* 58, 1212–1226. doi: 10.1016/j.dsr.2011.09.004

Höper, J., and Jahn, H. (1995). Influence of Environmental Oxygen Concentration on Growth and Vascular Density of the Area vasculosa in Chick Embryos. *Int. J. Microcircul.* 15, 186–192. doi: 10.1159/000178974

Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laughner, E., Wenger, R. H., et al. (1998). Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1α. *Genes Devel.* 12, 149–162. doi: 10.1101/gad.12.2.149

Kayar, S. R., Snyder, G. K., Birchard, G. F., and Black, C. P. (1981). Oxygen permeability of the shell and membranes of chicken eggs during development. *Respir. Physiol.* 46, 209–221. doi: 10.1016/0034-5687(81)90122-5

Kranenbarg, S., Van Den Boogaart, J. G. M., and Van Leeuwen, J. L. (2003). Oxygen profile in zebra fish embryo (Danio rerio) elucidated by theory and experiment. *Anim. Biol.* 53, 339–346. doi: 10.1163/157075603322556256

Kučera, P., Raddatz, E., and Baroffio, A. (1984). "Oxygen and glucose uptakes in the early chick embryo," in *Respiration and metabolism of embryonic vertebrates: Satellite Symposium of the 29th International Congress of Physiological Sciences, Sydney, Australia, 1983*, ed. R. S. Seymour (Dordrecht: Springer), 299–309. doi: 10.1007/978-94-009-6536-2_21

Künzel, W., Hohmann, M., and Braems, G. (1992). "Oxygen Deficiency During Fetal Development and Delivery — Speculations About Clinical Conditions," in *OXYGEN: Basis of the Regulation of Vital Functions in the Fetus*, eds W. Künzel and M. Kirschbaum (Berlin, Heidelberg: Springer), doi: 10.1007/978-3-642-77469-0_4

Kutchai, H., and Steen, J. B. (1971). Permeability of the shell and shell membranes of hens' eggs during development. *Respir. Physiol.* 11, 265–278. doi: 10.1016/0034-5687(71)90001-6

Lomholt, J. P. (1984). "A preliminary study of local oxygen tensions inside bird eggs and gas exchange during early stages of embryonic development," in *Respiration and metabolism of embryonic vertebrates: Satellite Symposium of the 29th International Congress of Physiological Sciences, Sydney, Australia, 1983*, ed. R. S. Seymour (Dordrecht: Springer), 289–298. doi: 10.1007/978-94-009-6536-2_20

Mantel, C. R., O'Leary, H. A., Chitteti, B. R., Huang, X., Cooper, S., Hangoc, G., et al. (2015). Enhancing Hematopoietic Stem Cell Transplantation Efficacy by Mitigating Oxygen Shock. *Cell* 161, 1553–1565. doi: 10.1016/j.cell.2015.04.054

Metcalfe, J., Mccutcheon, I. E., Francisco, D. L., Metzenberg, A. B., and Welch, J. E. (1981). Oxygen availability and growth of the chick embryo. *Respir. Physiol.* 46, 81–88. doi: 10.1016/0034-5687(81)90091-8

Meuer, H. J., and Baumann, R. (1988). Oxygen pressure in intra- and extraembryonic blood vessels of early chick embryo. *Respir. Physiol.* 71, 331–341. doi: 10.1016/0034-5687(88)90026-6

Meuer, H.-J., Hartmann, V., and Jopp, S. (1992). Tissue PO2 and growth rate in early chick embryos. *Respir. Physiol.* 90, 227–237. doi: 10.1016/0034-5687(92)90083-9

Mohlin, S., Wigerup, C., Jögi, A., and Påhlman, S. (2017). Hypoxia, pseudohypoxia and cellular differentiation. *Exp. Cell Res.* 356, 192–196. doi: 10.1016/j.yexcr.2017.03.007

Mortola, J. P. (2009). Gas exchange in avian embryos and hatchlings. *Mole. Integr. Physiol.* 153, 359–377. doi: 10.1016/j.cbpa.2009.02.041

Naňka, O., Valášek, P., Dvořáková, M., and Grim, M. (2006). Experimental hypoxia and embryonic angiogenesis†. *Devel. Dynam.* 235, 723–733. doi: 10.1002/dvdy.20689

Niklasson, C. U., Fredlund, E., Monni, E., Lindvall, J. M., Kokaia, Z., Hammarlund, E. U., et al. (2020). Hypoxia inducible factor-2α importance for migration, proliferation, and self-renewal of trunk neural crest cells. *Devel. Dynam.* 250, 191–236. doi: 10.1002/dvdy.253

Ota, K., Nagai, H., and Sheng, G. (2007). Expression and hypoxic regulation of hif1alpha and hif2alpha during early blood and endothelial cell differentiation in chick. *Gene Exp. GEP* 7, 761–766. doi: 10.1016/j.modgep.2007.05.007

Paganelli, C. V., Ar, A., Rahn, H., and Wangensteen, O. D. (1975). Diffusion in the gas phase: The effects of ambient pressure and gas composition. *Respir. Physiol.* 25, 247–258. doi: 10.1016/0034-5687(75)90001-8

Pelster, B., and Burggren, W. W. (1996). Disruption of Hemoglobin Oxygen Transport Does Not Impact Oxygen-Dependent Physiological Processes in Developing Embryos of Zebra Fish (Danio rerio). *Circul. Res.* 79, 358–362. doi: 10.1161/01.res.79.2.358

Rahn, H., and Paganelli, C. V. (1990). Gas fluxes in avian eggs: driving forces and the pathway for exchange. *Comp. Biochem. Physiol. Part A Physiol.* 95, 1–15. doi: 10.1016/0300-9629(90)90002-A

Rahn, H., Paganelli, C. V., and Ar, A. (1975). Relation of Avian Egg Weight to Body Weight. *Auk* 92, 750–765. doi: 10.2307/4084786

Revsbech, N. P. (1989). An oxygen microsensor with a guard cathode. *Limnol. Oceanogr.* 34, 474–478. doi: 10.4319/lo.1989.34.2.0474

Scully, D., Keane, E., Batt, E., Karunakaran, P., Higgins, D. F., and Itasaki, N. (2016). Hypoxia promotes production of neural crest cells in the embryonic head. *Development* 143, 1742–1752. doi: 10.1242/dev.131912

Semenza, G. L. (2012). Hypoxia-inducible factors in physiology and medicine. *Cell* 148, 399–408. doi: 10.1016/j.cell.2012.01.021

Seymour, R. S., and Visschedijk, A. H. J. (1988). Effects of variation in total and regional shell conductance on air cell gas tensions and regional gas exchange in chicken eggs. *J. Comp. Physiol. B* 158, 229–236. doi: 10.1007/bf01075837

Simon, M. C., and Keith, B. (2008). The role of oxygen availability in embryonic development and stem cell function. *Nat. Rev. Mol. Cell Biol.* 9, 285–296. doi: 10.1038/nrm2354

Tazawa, H., Wakayama, H., Turner, J. S., and Paganelli, C. V. (1988). Metabolic compensation for gradual cooling in developing chick embryos. *Compar. Biochem. Physiol. Part A* 89, 125–129. doi: 10.1016/0300-9629(88)91068-7

Tian, H., Hammer, R. E., Matsumoto, A. M., Russell, D. W., and Mcknight, S. L. (1998). The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Devel.* 12, 3320–3324. doi: 10.1101/gad.12.21.3320

Vaapil, M., Helczynska, K., Villadsen, R., Petersen, O. W., Johansson, E., Beckman, S., et al. (2012). Hypoxic Conditions Induce a Cancer-Like Phenotype in Human Breast Epithelial Cells. *PLoS One* 7:e46543. doi: 10.1371/journal.pone.0046543

Visschedijk, A. H. J., Ar, A., Rahn, H., and Piiper, J. (1980). The independent effects of atmospheric pressure and oxygen partial pressure on gas exchange of the chicken embryo. *Respir. Physiol.* 39, 33–44. doi: 10.1016/0034-5687(80)90012-2

Wagner-Amos, K., and Seymour, R. S. (2002). Effect of regional changes to shell conductance on oxygen consumption and growth of chicken embryos. *Respir. Physiol.* 129, 385–395. doi: 10.1016/s0034-5687(01)00320-6

Wangensteen, O., Wilson, D., and Rahn, H. (1970/71). Diffusion of gases across the shell of the hen's egg. *Respir. Physiol.* 11, 16–30. doi: 10.1016/0034-5687(70)90099-x

Wangensteen, O. D., and Rahn, H. (1970). Respiratory gas exchange by the avian embryo. *Respir. Physiol.* 11, 31–45. doi: 10.1016/0034-5687(70)90100-3

Yoshizaki, N., and Saito, H. (2002). Changes in Shell Membranes During the Development of Quail Embryos. *Poul. Sci.* 81, 246–251. doi: 10.1093/ps/81.2.246

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 23 June 2021
doi: 10.3389/fevo.2021.693781

# Starvation and Climate Change—How to Constrain Cancer Cell Epigenetic Diversity and Adaptability to Enhance Treatment Efficacy

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Advanced metastatic cancer is currently not curable and the major barrier to eliminating the disease in patients is the resistance of subpopulations of tumor cells to drug treatments. These resistant subpopulations can arise stochastically among the billions of tumor cells in a patient or emerge over time during therapy due to adaptive mechanisms and the selective pressures of drug therapies. Epigenetic mechanisms play important roles in tumor cell diversity and adaptability, and are regulated by metabolic pathways. Here, I discuss knowledge from ecology, evolution, infectious disease, species extinction, metabolism and epigenetics to synthesize a roadmap to a clinically feasible approach to help homogenize tumor cells and, in combination with drug treatments, drive their extinction. Specifically, cycles of starvation and hyperthermia could help synchronize tumor cells and constrain epigenetic diversity and adaptability by limiting substrates and impairing the activity of chromatin modifying enzymes. Hyperthermia could also help prevent cancer cells from entering dangerous hibernation-like states. I propose steps to a treatment paradigm to help drive cancer extinction that builds on the successes of fasting, hyperthermia and immunotherapy and is achievable in patients. Finally, I highlight the many unknowns, opportunities for discovery and that stochastic gene and allele level epigenetic mechanisms pose a major barrier to cancer extinction that warrants deeper investigation.

**Keywords: epigenetics, evolution, cancer, fasting, fever, stochastic gene expression, metabolism, adaptive therapy**

OPEN ACCESS

***Edited by:***
*Christopher J. Whelan, Moffitt Cancer Center, United States*

***Reviewed by:***
*Andriy Marusyk, Moffitt Cancer Center, United States*
*Benjamin Hopkins, Icahn School of Medicine at Mount Sinai, United States*

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***Specialty section:***
*This article was submitted to Population, Community, and Ecosystem Dynamics, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *12 April 2021*
***Accepted:*** *02 June 2021*
***Published:*** *23 June 2021*

***Citation:***
*Gregg C (2021) Starvation and Climate Change—How to Constrain Cancer Cell Epigenetic Diversity and Adaptability to Enhance Treatment Efficacy. Front. Ecol. Evol. 9:693781. doi: 10.3389/fevo.2021.693781*

## INTRODUCTION

Cancer is a disease that results from fundamental biological processes and mechanisms that enable diversity, adaptation and evolution (Merlo et al., 2006; Maley et al., 2017; McGranahan and Swanton, 2017). Stage IV metastatic cancer is currently not curable, and clinical treatment regimens are typically palliative, aiming to maximize patient quality and duration of life. Malignant cells arise most frequently in tissues with high cell division rates and from cell populations that have the capacity for cell division (i.e., reproduction) (Tomasetti et al., 2017). Tumor cell populations

in a single 1 $cm^3$ tumor can reach $10^7$–$10^9$ cells, with a doubling rate of every ∼15 days for a rapidly growing tumor and every 100 or more days for a slow growing tumor (Tubiana, 1989; Del Monte, 2009). Thus, a metastatic patient with multiple lesions and circulating tumor cells can have tens of billions of actively dividing malignant cells. With an average cell cycle time of ∼48 h and mutation rate of 1.14 mutations per genome per cell division (Werner et al., 2019), every gene in the cancer cell genome is affected by coding and non-coding genetic mutations multiple independent times in a patient with years of metastatic disease. All enemies are at the gate. Nonetheless, cancer cell evolution converges on a handful of specific driver mutations that are the major genetic drivers of malignancy (McGranahan et al., 2015; Tomasetti et al., 2015; Tokheim et al., 2016; Reiter et al., 2018). This observation led to the proposal that a curative combinatorial treatment strategy attacking these driver mutations could be developed (Reiter et al., 2019). However, while the identification of core genetic driver mutations in cancer is foundational and exciting, understanding epigenetic mechanisms driving cancer cell diversity and adaptability is another major barrier. Epigenetic mechanisms contribute to the evolution of multi-drug resistance (MDR), preventing us from curing metastatic cancer (Easwaran et al., 2014; Baylin and Jones, 2016; McGranahan and Swanton, 2017; Guo et al., 2019). The development of cancer cell subpopulations with MDR, or the ability to enter dormant, persistent states that evade treatment and immune predation, ultimately lead to disease progression and patient death (Recasens and Munoz, 2019; Shen et al., 2020). No single drug, new or old, will ever overcome these evolutionary forces and cure the disease. Currently, many chemotherapies and endocrine therapies target the *reproductive* capabilities of cancer cells by affecting cell division processes or signaling pathways that control cell division and tumor growth. Immunotherapies are distinct in that they enhance immune cell predation on cancer cells. However, the field lacks interventions aimed at solving the fundamental problem of how to constrain cancer cell diversity, adaptability and evolvability. Interventions that could homogenize cancer cell populations and constrain adaptability would help enable a chance at a cure using existing drugs.

Recent articles propose innovative treatment regimens for curing metastatic cancer that are inspired by the factors that drive the extinction of species in nature (Gatenby and Brown, 2018; Gatenby et al., 2019, 2020; Reed et al., 2020). Species extinctions often involve such complex interactions between unrelated stressors, rather than single catastrophic events. Central to these ideas are the application of aggressive, unpredictable, successive and combinatorial chemo, endocrine and immunotherapy treatment strikes that fragment cell populations and continue even after the cancer becomes clinically undetectable. Combinatorial approaches that are designed to be curative would benefit from interventions that help constrain cancer cell diversity and adaptability during drug treatment strikes. Here, I discuss how epigenetic gene regulatory mechanisms and allele-specific expression effects create cellular diversity and enable adaptability in cancer, creating barriers to its extinction. I highlight opportunities to learn from ecology, evolution, biochemistry, metabolism, genomics and conserved responses to bacterial infection to develop clinically relevant strategies to constrain cancer cell diversity and adaptability. My goal is to lay the conceptual groundwork and areas for further study to devise feasible treatment programs to make cancer cell populations more vulnerable to combinatorial chemotherapy strikes designed to cure metastatic disease.

## STOCHASTIC GENE REGULATORY EFFECTS ARE IMPORTANT DRIVERS OF CELLULAR DIVERSITY AND ADAPTABILITY

Previous decades have largely focused on cancer genetics and the identification of important genetic mutations. Less is known about gene regulatory and epigenetic mechanisms in cancer, though it is emerging as a major field of study and a new area for therapy development. From ecology and species evolution, we know that protein-coding genes are relatively well conserved across species, while gene regulatory mechanisms and *cis*-regulatory elements (CREs) are rapidly evolving. Changes to gene regulatory network (GRNs) play the major roles in the development of new phenotypes in different lineages (Davidson and Erwin, 2006; Wray, 2007; Carroll, 2008; Davidson, 2010). Recent reviews have covered the emerging and likely important roles that epigenetic mechanisms also play in cancer initiation and progression, metastasis, and drug resistance (Guo et al., 2019), as well as opportunities for targeting these mechanisms to treat the disease (Bennett and Licht, 2018; Cheng et al., 2019; Hogg et al., 2020). Epigenetic mechanisms and stochastic changes to GRNs and gene expression enable a dynamic range of phenotypic possibilities for a population of cells or organisms.

Pioneering studies in bacteria first showed that isogenic cells held in constant conditions occupy a wide-range of different states due to stochastic gene expression and transcriptional bursts (Li, 2002; Ozbudak et al., 2002). Subsequent studies of eukaryotic cells reached similar conclusions (Blake et al., 2003). It is now well established that, at baseline, cells exist in flux, creating molecularly diverse populations through stochastic gene expression (Kærn et al., 2005; Levine et al., 2013), transcriptional bursting and transitions between active, reversibly silent and irreversibly silent chromatin states (Singer et al., 2014; Bintu et al., 2016; **Figure 1**). This has the important effect of creating diversity for adaptability and "bet hedging" so that at least some cells are in the right molecular state to receive and correctly respond to unpredictable signals from the environment (Raj and van Oudenaarden, 2008; Feinberg and Irizarry, 2010; Raj et al., 2010; Balázsi et al., 2011). In the context of stressors, this diversity may help prokaryotic and eukaryotic cells survive acute insults by helping to ensure that at least some cells exist in a state that is resilient to the stressor.

This type of stochastic epigenetic cellular diversity has been proposed to have important roles in cancer evolution and drug resistance (Pujadas and Feinberg, 2012). However, we currently have little understanding of the mechanisms involved or how

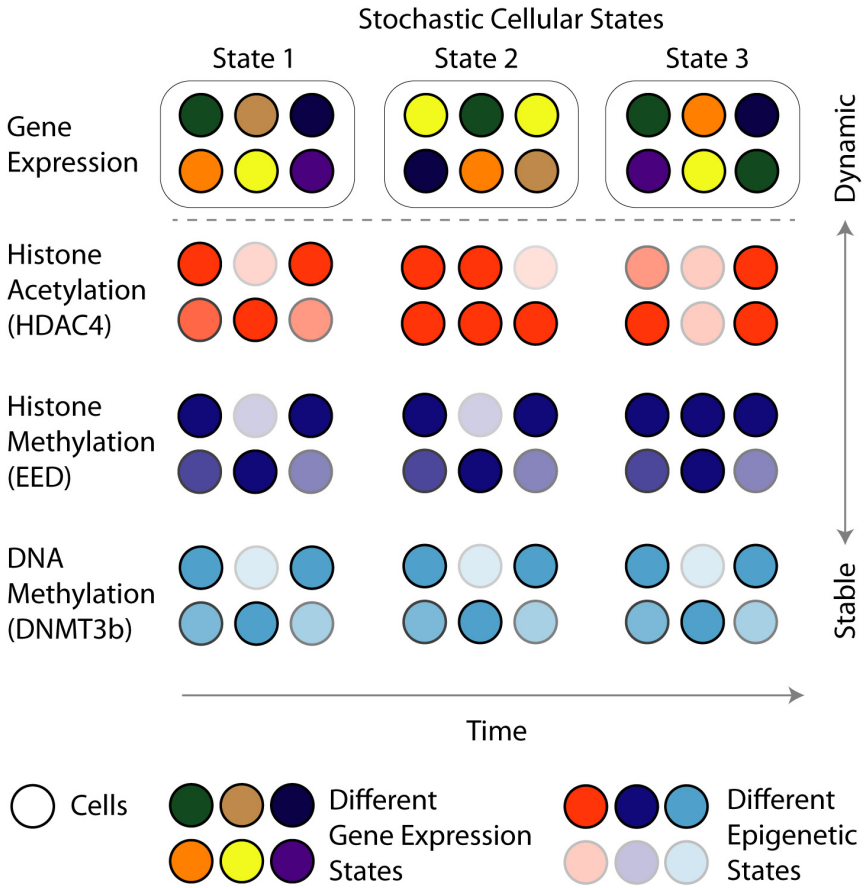


**FIGURE 1 |** Schematic depiction of how stochastic gene expression and epigenetic regulation creates mosaics of cells in different molecular states. Studies by Bintu et al. (2016) show that histone acetylation is dynamic and mediates short-term epigenetic memory, while histone and DNA methylation is more stable, mediating long-term epigenetic memory. These stochastic gene expression and epigenetic effects contribute to cellular diversity and may enable enhanced tumor cell diversity and adaptability.

to modulate or constrain the effect. Few studies have directly examined chromatin dynamics at the cellular level over time. Chromatin biochemical modifications are diverse and new forms are constantly being discovered. Major epigenetic mechanisms involved in stochastic epigenetic diversity likely include histone acetylation, DNA methylation and histone methylation. A brief overview of these mechanisms is described below, but I refer the reader to excellent recent reviews for further detail (Campbell and Wellen, 2018; Dai et al., 2020; Trefely et al., 2020).

In brief, histone tails are biochemically modified post-translationally to regulate gene expression. The chromatin modifiers (writers) that establish these marks use metabolic intermediate molecules, including acetyl-CoA and S-adenosylmethionine (SAM). Acetyl-CoA is the metabolite used by histone acetyltransferases (HATs) to place acetyl groups on lysine residues of the N-terminal tails of H3 and H4 canonical histones. Acetyl groups are a subtype of acyl organic molecules distinguished by the inclusion of a –CH3 group. In the absence of acetylation, the positive charges on H3 and H4 histones combine with the negative charge on the surface of H2A histone fold domains to enable the formation of nucleosomes and compact chromatin. One effect of H3 and H4 lysine acetylation is to change the overall histone charge to neutral, which reduces histone affinity, creating open chromatin sites in which transcriptional regulatory proteins can bind. The creation of these open chromatin sites through lysine acetylation is important for the activation of non-coding enhancers, gene promoters, gene bodies and alternative exon usage through splicing variation (Rajagopal et al., 2014). In addition to promoting open chromatin states, bromo-domain containing chromatin "reader" proteins recognize lysine acetylation and bind to promote gene expression. Recently, other histone acylations have been uncovered, which also have activating effects on gene expression (Dai et al., 2020). On the other hand, histone deacetylation by histone deacetylases (HDACs) promotes gene silencing and the formation of heterochromatin. Acetyl groups are added to histone tails by HATs, which are divided into three groups, including GNAT, MYST, and p300/CBP. The HDACs that remove acetyl groups in mammals are divided into 4 groups, including the zinc-dependent class I, II, and IV HDACs, and the NAD-dependent class III HDACs, which are also known as sirtuins.

In addition to Acetyl-CoA dependent chromatin acetylation dynamics, SAM dependent chromatin methylation dynamics are primary players in orchestrating cellular epigenetic states and GRNs. De novo DNA methylation is performed by DNMT (DNA methyltransferase) 3a and DNMT3b, and maintenance of DNA methylation is performed by DNMT1, which recognizes hemi-methylated DNA. DNA methylation frequently occurs on cytosines located in sets of CpG dinucleotide repeats called CpG islands, which are located near transcription start sites. Methylation of these regions contributes to gene silencing. Removal of the silencing can be achieved by active DNA demethylation, which is primarily regulated by the TET family of DNA demethylase enzymes, as well as by passive DNA demethylation due do inhibition of DNMT1 in dividing cells, which causes the methylation mark to be lost over successive cell divisions.

Methylation also occurs on histone tails, including lysine and arginine residues, which alters the affinity of histone-methylation reader proteins to bind and affect gene expression. Some forms of histone methylation are associated with gene activation, such as H3K4, H3K79, or H3K36 methylation. On the other hand, H3K9, H3K27, and H4K20 methylation are associated with gene silencing and different methylation states, including mono-, di- or tri- methylation of the same amino acid residue can have different effects on gene expression. Histone methylation is mediated by histone methyltransferases. For example, the EZH2 (enhancer of zeste 2) enzymatic subunit of the polycomb repressor complex 2 (PRC2) catalyzes the formation of H3K27me3, while KRAB (Kruppel associated box) domain containing zinc finger protein transcription factors can catalyze the formation of H3K9me3. Both of these chromatin modifications have potent silencing effects and important roles in the formation of stable heterochromatin.

Building on this understanding of epigenetic gene regulation, a pioneering study by Bintu et al. created an elegant reporter assay to study the temporal dynamics of histone acetylation, DNA methylation and histone methylation at the cellular level (Bintu et al., 2016). They compared the cellular repression induction and reactivation kinetics for DNA methylation (DNMT3B), H3K9me3 (KRAB), H3K27me3 (EED-EZH2 component of PRC2) and H3/H4 histone deacetylation (HDAC4). Their results show that DNMT3B, KRAB, and EED-EZH2 induce stable chromatin changes that cause permanent epigenetic memory. DNMT3B in particular showed slow induction kinetics, but caused stable and permanent epigenetic memory in affected cells. These results are consistent with previous work showing that DNA methylation is a relatively stable biochemical mark. In contrast, HDAC4 effects are highly dynamic, such that activation of HDAC4 caused the induction of faster chromatin changes than the other enzymes tested and the effects are transient and rapidly reversible. The rapid and dynamic roles of histone acetylation in these hamster ovary cell lines are consistent with previous work in yeast, which showed that histone acetylation and deacetylation states can switch within minutes (Katan-Khaykovich and Struhl, 2002). Overall, these results suggest that reducing the enzymatic activity of HATs and HDACs could constrain short-term, dynamic stochastic cellular diversity and adaptability. Further, reducing the activity of enzymes controlling DNA and histone methylation could help constrain long-term, stable stochastic cellular diversity and adaptability (**Figure 1**). Below, I next discuss how stochastic epigenetic effects not only occur at the gene level, but also at the allele level, which potentially further create primary epigenetic and gene expression barriers to cancer "extinction."

## STOCHASTIC GENE REGULATORY EFFECTS AT THE ALLELE LEVEL AS A POTENTIAL DRIVER OF TUMOR CELL DIVERSITY AND ADAPTABILITY

Stochastic gene regulatory effects also occur at the allele level, which could further contribute to cancer cell diversity and evolvability. Indeed, the diploid and, in some cases, polyploid nature of eukaryotic cells creates added cellular epigenetic, gene expression and genetic diversity. The advantage of diploidy over haploidy has typically been proposed to be to mask the effects of partially recessive mutations (Orr, 1995). Interestingly, however, periods associated with catastrophic extinction events, such as the comet or asteroid strike and volcanic eruptions at the Cretaceous–Paleogene boundary, were previously shown to be associated with evolutionary bursts of new species with whole genome duplication events (Madlung, 2012; Van de Peer et al., 2017). In other words, increased ploidy was associated with improved survival during a mass extinction event. Polyploidy is proposed to have been beneficial because it increases allelic diversity and species adaptability (Fox et al., 2020). In mammals, most cells are diploid, though specialized cell-types, such as liver hepatocytes, are polyploid. It has been observed that normal diploid cells in the body can increase their ploidy in response to stress (Fox et al., 2020). For cancer, tumor cell acquisition of polyploidy or aneuploidy are major features of the disease that are thought to contribute to rapid tumor evolution (Krajcovic and Overholtzer, 2012; Coward and Harding, 2014). Typically, increases in ploidy are considered to drive increased genetic variation, however, through allele-specific epigenetic regulatory effects, increased ploidy could also promote increased *epigenetic* variation (**Figure 2**).

Previous *in vitro* studies of cell lines have suggested the existence of random monoallelic expression (RME) for thousands of autosomal genes in mice and humans due to non-genetic mechanisms (Gimelbrant et al., 2007; Eckersley-Maslin et al., 2014; Gendrel et al., 2014). RME in cell lines is mitotically heritable and has been shown to be regulated by levels of the insulator protein, CTCF, in some cases (Chandradoss et al., 2020). However, the prevalence of widespread autosomal RME that is clonal (mitotically heritable) is currently debated and little is known about the existence of such effects *in vivo* (Reinius and Sandberg, 2015; Rv et al., 2021; Vigneau et al., 2018). Some have suggested that bona fide clonal RME is rare on the autosomes and that most observed cases are linked to transcriptional bursting and low expression (Deng et al., 2014; Reinius et al., 2016; Larsson et al., 2018; Symmons et al., 2019). Nonetheless, stochastic

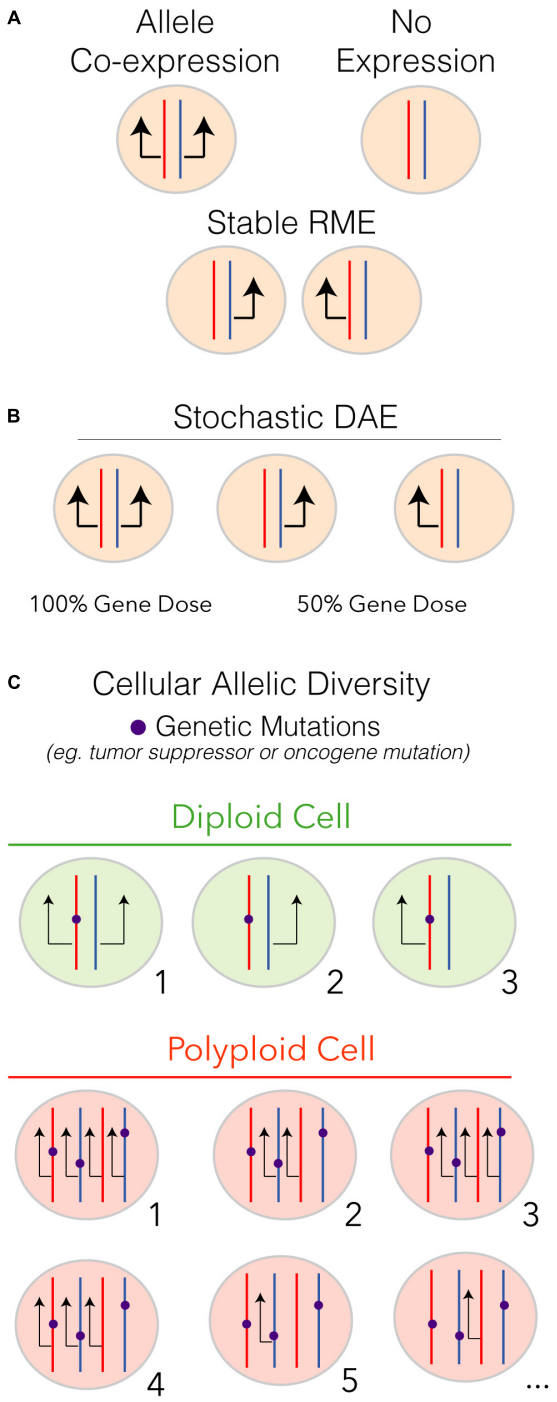


**FIGURE 2 |** Allele-specific epigenetic and expression create complex and diverse cellular states. **(A)** Classic biallelic (allele co-expression) and no expression states typically associated with autosomal genes, and clonal random monoallelic states (RME) typically associated with random X-inactivation in females. **(B)** Newly described stochastic differential allele expression (DAE), which causes biallelic and stochastic allelic expression states at the cellular level for thousands of autosomal genes in mice. **(C)** DAE interacts with heterozygous mutations in diploid and polyploidy cells to create widespread cellular diversity and potential for adaptability to different conditions. This phenomenon could be a major blocker to cancer cell extinction.

allele-specific expression for some autosomal genes appears stable at the cellular level. For example, one study directly imaged allelic expression at the cellular level over time for *Bcl11b* in T cell lineages (Ng et al., 2018). The authors found that monoallelic (or biallelic) expression states can be stable at the protein level for at least one hundred hours in single cells, amounting to several days (Ng et al., 2018). This type of allele-specific regulatory effect might be sufficient to have biological consequences, affecting gene dosage and/or the cellular effects of a single mutated allele.

Focusing on the *in vivo* context, we previously uncovered hundreds of genes in mouse tissues that display stochastic differential allelic expression (DAE) at the cellular level (Huang et al., 2017). We found that DAE affects the cellular expression of heterozygous mutations such that mosaics of mixed cells arise in tissues in which some cells monoallelically express the mutated allele, some express the wild-type allele and others are biallelic and express both parental alleles (Huang et al., 2017). Stochastic DAE may be an important feature of diploid (or polyploidy) genomes that increases cellular diversity and adaptability to stress (Huang et al., 2018; Kravitz and Gregg, 2019).

With regard to nomenclature, we differentiate stochastic DAE from bona fide RME, because RME is typically used to describe clonal, mitotically-heritable and stable *monoallelic* states, such as random X-inactivation, olfactory receptor or protocadherin monoallelic expression states (Lomvardas and Maniatis, 2016; Monahan et al., 2019; **Figures 2A**, **3**). In the case of stochastic DAE, we found that some cells are monoallelic and others are biallelic, and the temporal stability of each allelic state and the clonal relationships between cells are not yet known (**Figure 2B**). Mechanistically, stochastic DAE and clonal RME likely involve different epigenetic mechanisms, including different roles for short-term (e.g., histone acetylation) versus long-term (e.g., methylation or CTCF) epigenetic memory (**Figure 3**). This new area is expected to reveal important allele-specific gene regulatory mechanisms that enable increased phenotypic variability and adaptable metabolic phenotypes (Huang et al., 2018; Kravitz and Gregg, 2019). Currently, we do not understand the nature of these different allelic effects in tumor cells or how they may change and contribute to tumor initiation, metastasis, evolution and drug resistance, and affect patient survival.

Given that polyploidy and aneuploidy are linked to cancer progression and evolution, and appear to predict worse outcomes (Coward and Harding, 2014; Krajcovic and Overholtzer, 2012), it is possible that cancer cells benefit from increases in ploidy by increasing cellular diversity through stochastic allele-specific epigenetic and gene expression effects. Previous studies found that progenitor versus differentiated cellular states are associated with dramatic differences in RME, suggesting these mechanisms have roles in defining different cellular proliferative versus differentiated states (Miyanari and Torres-Padilla, 2013; Eckersley-Maslin et al., 2014; Gendrel et al., 2014; Jeffries et al., 2016; Branciamore et al., 2018; Ng et al., 2018). Recent studies have also begun to show how stochastic allele-specific epigenetic effects can interact with allele-specific genetic variants to create allelic diversity (Onuchic et al., 2018; Zhang S. et al., 2020). In summary, the studies above show the enormous potential for stochastic epigenetic and gene expression effects to drive cellular

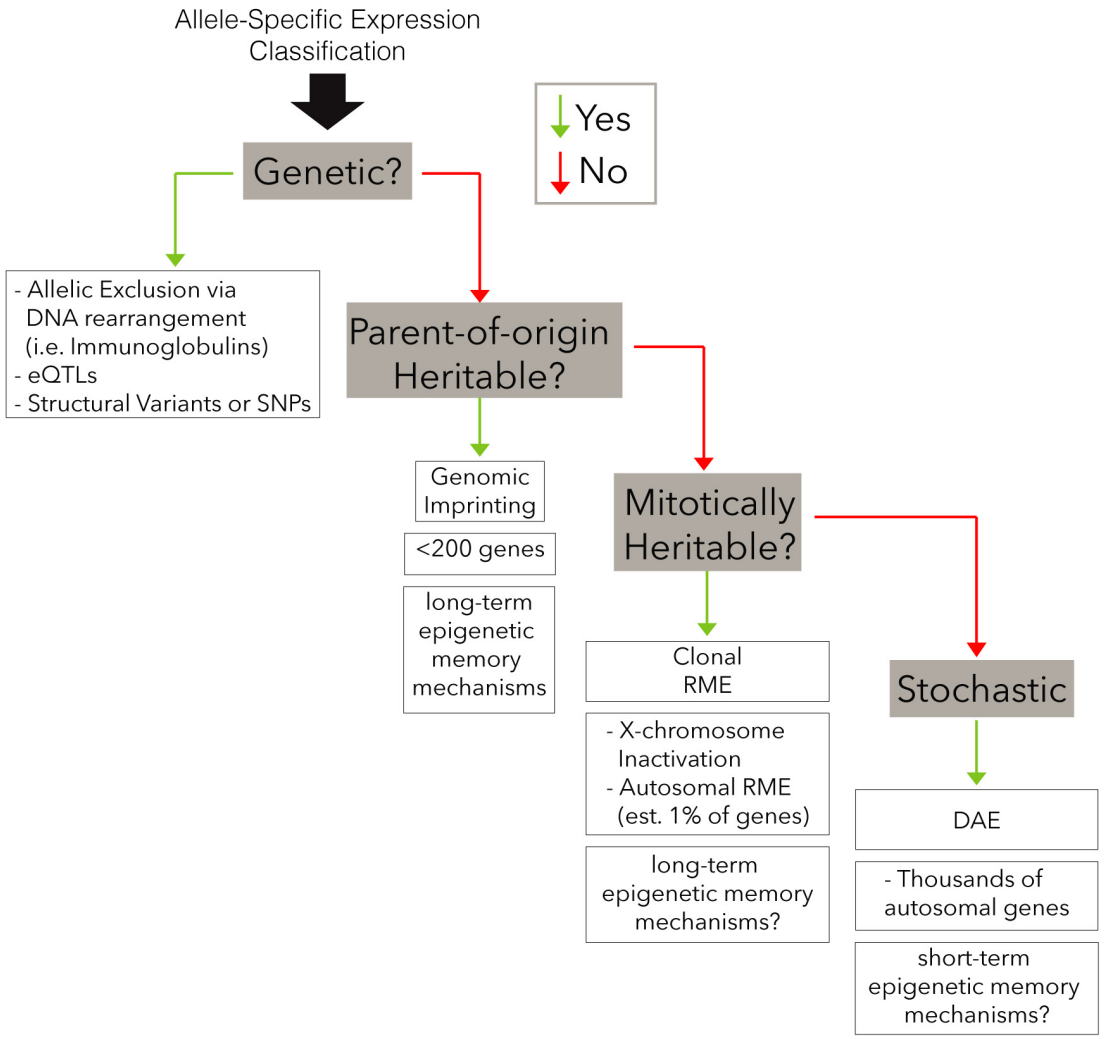


**FIGURE 3 |** A decision classification tree for different subtypes of allele-specific epigenetic and expression effects according to heritability, clonality and stability. The decision tree shows that different allele-specific expression effects arise according to genetic, genomic imprinting, clonal RME and stochastic DAE. Each of these increases cellular diversity and adaptability and are potential barriers to eliminating metastatic cancer in patients.

diversity in cancer and the different potential forms of these effects at the gene and allele levels. Next, I discuss findings that show that stochastic epigenetic effects are, in fact, the primary drivers of tumor cell evolution and drug resistance, which motivates discussions for new studies and clinical solutions.

## HOW DOES STOCHASTIC EPIGENETIC VARIATION ENABLE CANCER CELL EVOLUTION?

Intra-tumor cellular epigenetic and gene expression heterogeneity is proposed play the primary roles in the acquisition of drug resistance in cancer compared genetic mutations (Flavahan et al., 2017; Marusyk et al., 2020). A leading model in the field is that stochastic and semi-stable changes to gene expression and chromatin states cause drug-resistant phenotypes to arise dynamically within tumor cell populations. In this model, stochastic epigenetic and phenotypic cellular diversity creates an ecosystem in which a drug treatment can "discover" a pre-existing tumor cell in a state that enables it to tolerate the drug and persist. This epigenetic state is therefore advantageous for survival and proliferation compared to other tumor cell states in the microenvironment. Not only will this cell survive, persist and continue to reproduce, but its advantageous chromatin state can become enhanced, stabilized and mitotically heritable through further induced chromatin and gene expression changes. This evolutionary process drives the formation of dangerous new lineages of drug resistant tumor cells.

Support for this model of tumor evolution and drug resistance is strong. Indeed, epigenetic stochasticity has been shown to be a central driver of cellular phenotypic variability and mechanism of plasticity (Jenkinson et al., 2017). A seminal paper by Sharma

et al. showed that individual tumor cells stochastically and transiently acquire and then relinquish chromatin-mediated drug resistant states (Sharma et al., 2010). Moreover, they showed that chromatin-modifying agents selectively ablated the resistant cell population. Subsequently, others found that the histone demethylases, KDM5A and KDM6B, regulate phenotypic heterogeneity in estrogen receptor positive breast cancer (Hinohara et al., 2018). High KDM5B activity promotes increased intra-tumor gene expression heterogeneity, creating pre-existing cell populations that increase chances for drug resistance (Hinohara et al., 2018). Further, studies of DNA methylation dynamics in chronic lymphocytic leukemia (CLL) found that stochastic epimutations form the basis of intratumor cellular methylome variability (Landau et al., 2014). More recently, single-cell DNA methylome analyses in CLL revealed that the cellular inheritance of stochastic DNA methylation epimutations reveal a lineage tree for the cellular evolution of the disease and that epigenetic drift occurs rapidly following B cell transformation and greater proliferation rates (Gaiti et al., 2019). Moreover, increases in stochastic epigenetic diversification in CLL appear to contribute to a larger diversification of gene expression and cellular molecular identities in the disease (Gaiti et al., 2019; Pastore et al., 2019). Thus, primary roles for stochastic and dynamic cellular epigenetic and gene expression variability in the selection, formation and evolution of drug resistant tumor cells are now well supported (Huang et al., 2009; Huang, 2013; Pisco et al., 2013; Knoechel et al., 2014; Flavahan et al., 2017; Liau et al., 2017; Shaffer et al., 2017; Risom et al., 2018; Marusyk et al., 2020). We now have a maturing conceptual framework for understanding "mutation-independent" evolution and the primary roles that epigenetic and gene expression diversity play in the development of new phenotypes (Huang et al., 2009; Huang, 2011, 2012, 2021). One of the most glaring facts supporting this model is that genetic mutations are not required for cells to "evolve" into different cell lineages during organismal development—this diversity is entirely grounded in epigenetic and gene expression changes (Huang, 2012).

The major implication of all of the findings in the above sections is that uncovering ways to constrain tumor cell epigenetic regulatory activity and dynamics will help constrain cancer cell diversity, adaptability and evolution during treatment. Placing constraints on cellular acetylation and methylation dynamics could be especially effective, but no single molecular target will solve this problem because of redundancy and adaptability. Next, I discuss clues for how to effectively constrain cellular epigenetic diversity and dynamics in a clinically relevant manner that could be feasibly integrated into a chemotherapy treatment regimen.

## EVOLUTIONARY SOLUTIONS TO STOCHASTIC POPULATION DIVERSITY AND ADAPTABILITY

Since both prokaryotic and eukaryotic cells use stochastic gene regulatory mechanisms and transcriptional noise for promoting cellular diversity and adaptability, we can potentially learn solutions to constrain these effects by analyzing how vertebrates evolved solutions to prokaryotic infections. The numbers of bacteria involved in an infection can exceed the population sizes that cancer cells reach in a body, yet the body can drive them to extinction. To achieve this, vertebrates evolved a highly conserved set of sickness responses that help to effectively eliminate infections in combination with activation of the immune system (Hart, 1988; Aubert, 1999; Dantzer and Kelley, 2007). This combinatorial response involves: (1) fasting (loss of appetite), (2) fever, (3) sleepiness and fatigue, (4) social withdrawal and irritability, and (5) altered motivations (inhibited foraging and exploration) (Hart, 1988; Aubert, 1999; Adelman and Martin, 2009). This adaptation for infection turns out to be highly relevant to cancer elimination. A history of the immunotherapy field reveals that interest in immune system predation on tumor cells began with early observations of tumors disappearing in patients following a bacterial infection with a high fever (Dobosz and Dzieciątkowski, 2019). Subsequently, William Coley showed that cancer patients enter spontaneous remission after a streptococcal skin infection (i.e., *erysipelas*). Moreover, bacterial infections can induce complete remission in several cancer types (Dobosz and Dzieciątkowski, 2019). So far, this work has largely inspired the development of targeted immunotherapies that aim to improve immune cell detection and killing of cancer cells, but there may be more to learn.

Vertebrate sickness responses are typically proposed to function for diverting energy from activities peripheral to surviving infection to immune responses that combat the infection (Hart, 1988; Aubert, 1999; Dantzer and Kelley, 2007). However, if the goal was only to increase resources for immune defense, namely boost the concentrations of the substrates and cofactors needed to support biochemical reactions for effective immunity, one might instead expect the animal to display increased appetite and caloric intake, rather than fasting. Recently, fasting, and the associated shift to ketone metabolism, was shown to reduce the damaging effects of reactive oxidative species (ROS) generated by bacterial inflammation, indicating an important function for this component of the sickness response that is different from the energy conservation model (Wang et al., 2016). However, as I discuss below, fasting may have additional benefits that involve constraining capabilities for stochastic cellular diversity and adaptability by limiting the availability of key substrates and cofactors.

From the perspective of preserving energy for the immune attack, coupling fasting with fever during sickness responses might seem to be counterproductive. How fever offers a protective mechanism against pathogenic microbes is a long-standing mystery (Evans et al., 2015). Fever is currently thought to create conditions that are inhospitable for microbe proliferation by raising the body's temperature above optimal growth conditions, while potentiating the immune response by increasing neutrophil activity and lymphocyte proliferation and activation (Hart, 1988; Evans et al., 2015). However, with the possible exception of unique immune cells, fever could constrain biochemical reaction kinetics in cells and thereby further constrain capabilities for creating a range of different

stochastic cellular states. By constraining the diversity and adaptability of microbes, immune cell predation would be more effective. As I discuss below, fever could especially help block dangerous hypometabolic hibernation-like states that enable disease persistence.

Thus, by *combining fasting and fever* with increased immune predation, the body has not only evolved an effective combinatorial strategy for driving the extinction of invading microbes, but apparently also cancer. We now know that nutrients and metabolic processes affect epigenetic gene regulatory mechanisms, suggesting an important link exists between fasting, fever and the epigenetic mechanisms that enable cellular diversity and adaptability. Noticeably, the vertebrate sickness response combines the starvation, climate change and predation conditions that frequently cause species extinctions in the wild. It is a highly effective recipe for this outcome.

## COMBINING FASTING AND HYPERTHERMIA TO CONSTRAIN CANCER CELL DIVERSITY AND ADAPTABILITY DURING CHEMOTHERAPY STRIKES

The benefits of fasting for cancer and other diseases have been carefully reviewed elsewhere (Nencioni et al., 2018; de Cabo and Mattson, 2019; Caffa et al., 2020; Tajan and Vousden, 2020; Zhang J. et al., 2020). Pre-clinical (Lee et al., 2012; Brandhorst et al., 2015; Wei et al., 2017; de Cabo and Mattson, 2019; Caffa et al., 2020), and early clinical studies (Caffa et al., 2020; de Groot et al., 2020), show benefits for coupling fasting or fasting-mimicking diets (FMDs) with chemotherapy and/or endocrine therapy, including enhanced treatment efficacy, reduce side effects and the prevention of drug resistance. The beneficial effects of fasting in cancer have so far been largely attributed to reductions in circulating glucose, insulin, IGF-1, PI3 kinase, mTOR and leptin signaling, which reduces growth signals that can drive cancer cell proliferation and survival. Beneficial effects of increased autophagy and stem cell activation are also apparent. Different durations and patterns of fasting induce different biological effects in a cell-type dependent manner. It has been suggested that the induction of a starvation response, in which cellular autophagy is strongly activated, is important for deriving anti-cancer benefits (Brandhorst et al., 2015; Nencioni et al., 2018). Important for this article, is that starvation and nutrient deprivation affect epigenetic mechanisms. Such effects could help constrain tumor cell diversity and adaptability. Starvation states can affect the availability of essential substrates and cofactors necessary for enzymatic modifications to chromatin and chromatin binding transcriptional regulatory proteins. Starvation states also block global protein translation by inhibiting mTOR complex 1 (mTORC1), which in turn limits cellular capabilities for intra and intercellular signaling and gene expression dynamics (Holcik and Sonenberg, 2005; Wullschleger et al., 2006). Indeed, starvation state translational and transcriptional programs involve a shift to specific stress response mechanisms that are essential for cell survival.

## POTENTIAL FOR STARVATION STATES TO CONSTRAIN STOCHASTIC DNA AND HISTONE METHYLATION AND LONG-TERM CELLULAR EPIGENETIC MEMORY

To my knowledge, no study has yet determined whether starvation reduces stochastic epigenetic dynamics within a cell over time, epigenetic diversity across populations of cells, or capabilities for epigenetic and gene expression adaptability in response to new and additional stressors (e.g., chemotherapy strikes). However, there are reasons to expect such effects. An enzyme's Km is the concentration of a substrate needed for the rate of its catalytic reaction to be half of the maximum rate (Vmax) and can further depend on the concentration of necessary cofactors. Relative to their Km, the physiological concentrations of the substrates and cofactors needed for HAT, HMT, and DNMT mediated chromatin-modifying reactions are low (Reid et al., 2017). As a result, the kinetics of histone acetylation and methylation biochemical reactions are sensitive to changes in these substrate concentrations and inhibited by reduced cellular concentrations of nutrient-derived cofactors and substrates (Su et al., 2016; Reid et al., 2017; **Figure 4A**). This differs from phosphorylation and ubiquitination reactions that are not as responsive to metabolic changes because their substrate, ATP, does not reach cellular levels low enough to limit enzymatic activity (Locasale and Cantley, 2011). Consequently, starvation limits the availability of essential SAM and Acetyl-CoA substrates needed for methylation and acetylation dynamics, respectively, while simultaneously activating nutrient stress response epigenetic and gene expression programs for survival. This may constrain the range of different epigenetic states cells can occupy, in addition to the other anti-cancer benefits of fasting/FMD/starvation (**Figure 4B**).

SAM is an essential cofactor for histone and DNA methyltransferases and is an intermediate of one-carbon metabolism derived from dietary methionine and synthesized through the methionine and folate metabolic cycles (**Figure 5**). Dietary methionine restriction causes significant alterations to cellular DNA methylation, histone methylation and gene expression (Mentch et al., 2015; Su et al., 2016), though we know less about effects on cellular epigenetic diversity or temporal dynamics. Recent work in mice found that a methionine-restricted diet rapidly altered methionine and sulfur metabolism, inhibiting tumor growth and increasing tumor susceptibility to chemotherapy and radiation (Gao et al., 2019). These results suggest decreased capabilities for tumor cells to adapt to the treatment strikes. However, further studies are needed to determine whether a methionine-restricted diet can help prevent the development of drug resistance either by reducing cellular epigenetic diversity and/or adaptability over time. Starvation or FMD cycles in mice have been shown to significantly improve

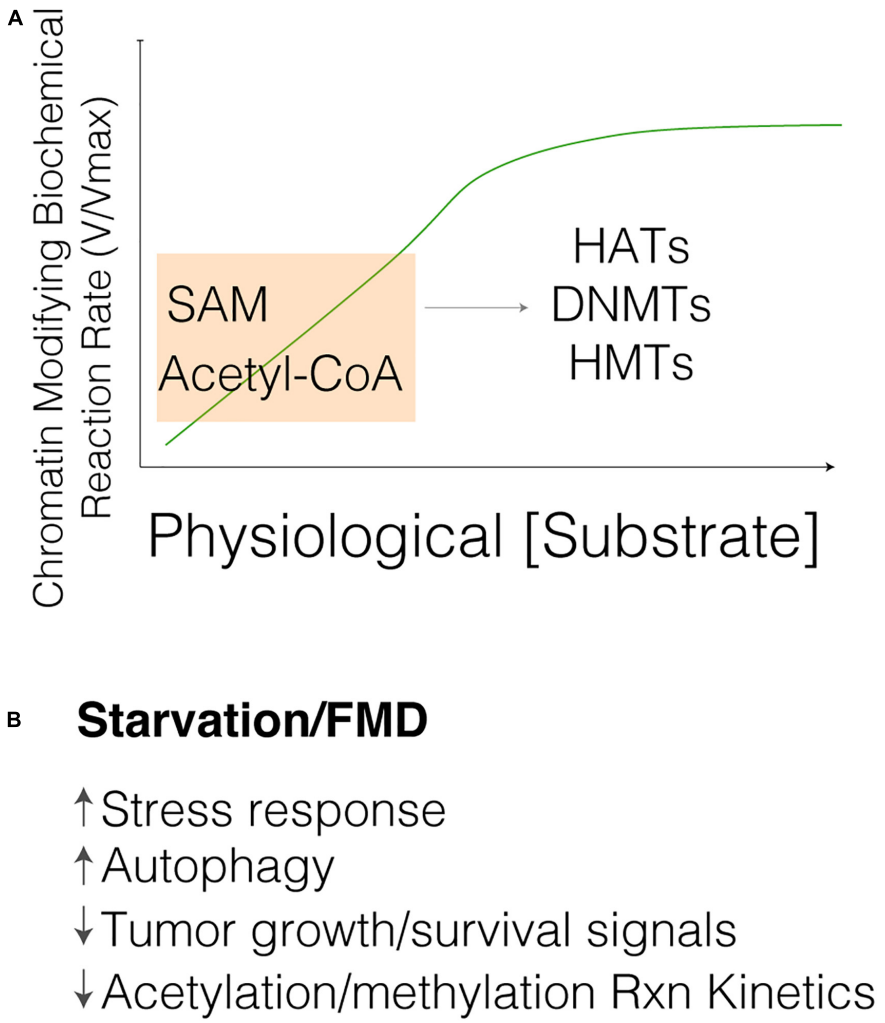


**FIGURE 4 |** Physiological concentrations of Acetyl-CoA and SAM are rate limiting for HATs, DNMTs, and HMTs and affect chromatin modifications. **(A)** Dietary changes that limit cellular SAM and Acetyl-CoA substrate concentrations are expected to reduce biochemical reactions mediating chromatin acetylation and methylation dynamics because these substrates lie within concentrations that are enzymatically rate limiting. **(B)** The hypothesized benefits of starvation periods or fasting-mimicking diets (FMD) are summarized and could include decreased acetylation and methylation reaction kinetics that help impair tumor cell diversity and adaptability.

survival, increase time to progression and even restore drug responsiveness to previously resistant tumors (Lee et al., 2012; Brandhorst et al., 2015; Caffa et al., 2020). Currently, we do not fully understand the mechanisms involved or to what degree methionine-restriction alone can achieve the benefits of starvation or FMD treatments.

## POTENTIAL FOR STARVATION STATES TO CONSTRAIN STOCHASTIC HISTONE ACETYLATION AND SHORT-TERM EPIGENETIC MEMORY

Methionine-restriction is not expected to directly alter histone acetylation dynamics. However, nutrient deprivation or inhibition of glycolysis causes significantly decreased acetyl-CoA levels, which in turn reduces histone acetylation (Wang et al., 2009; Lee et al., 2014; Mariño et al., 2014; Cluntun et al., 2015). Acetyl-CoA abundance is a key factor controlling gene expression by affecting chromatin structure to create open chromatin sites for gene activation. In addition, Acetyl-CoA abundance affects the acetylation of transcription factors by altering their stability, subcellular localization, or abilities to bind to DNA (Choudhary et al., 2014). Acetyl-CoA is produced from pyruvate through the tricarboxylic acid (TCA) cycle or by beta-oxidation of fatty acids (Campbell and Wellen, 2018). Little is known about the effects of starvation, fasting or FMD on histone acetylation dynamics at the cellular level or over time within a cell. One study tested whether a high fat diet (HFD) affects acetyl-CoA levels and global histone acetylation in mice, uncovering tissue specific effects after a 4 week HFD (Carrer et al., 2017). They found that

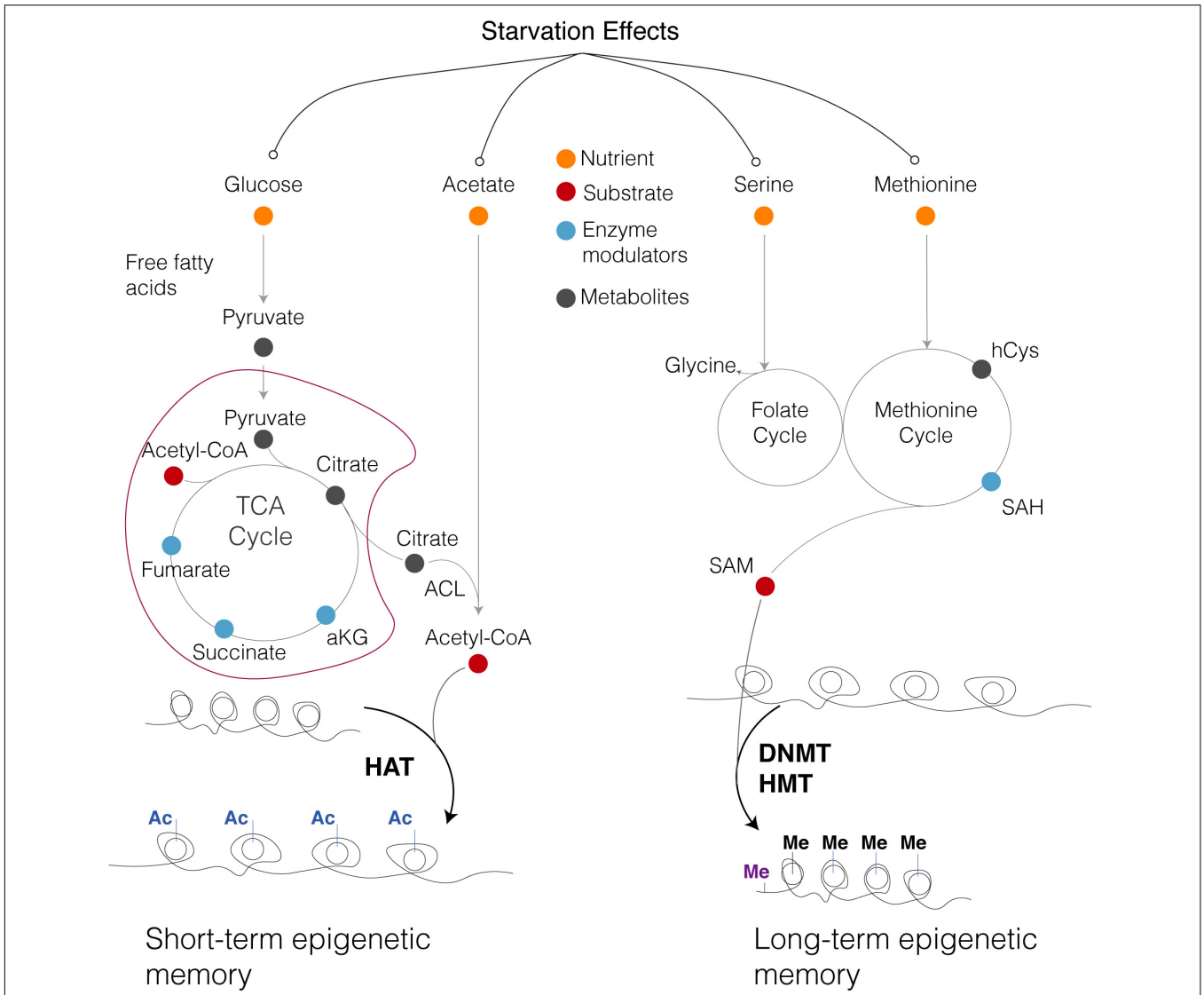


**FIGURE 5 |** A summary of how starvation-triggered nutrient deprivations converge on epigenetic pathways for short-term and long-term epigenetic memory. Nutrient deprivations that affect glucose, acetate, serine and methionine levels are predicted to impact the efficiency of cellular chromatin modifying reactions. Acteyl-CoA reductions are expected to most strongly affect epigenetic dynamics for short-term memory, while changes to methionine and SAM will affect epigenetic mechanisms mediating longer-term epigenetic effects. Adapted from Reid et al. (2017).

white adipose tissue showed significantly decreased Acetyl-CoA and histone acetylation for specific lysine residues. Thus, diet can affect global histone acetylation in a tissue dependent manner (**Figure 5**).

AMP-activated protein kinase (AMPK) is phosphorylated in response to starvation and inhibits anabolic glucose, lipid and protein synthesis pathways, and activates autophagy and mitophagy for the breakdown of cellular macromolecules (Herzig and Shaw, 2017). Cellular lipid stores are consumed through lipid metabolic pathways and, in an attempt to increase glucose uptake, AMPK promotes increased glucose transporter functions. Along with this energy stress response, AMPK inhibits the HAT, p300, and glucagon release during fasting causing the activation of class II HDACs and localization to the nucleus to activate transcriptional stress responses. Finally, sirtuins are activated in response to nutrient stress and use NAD+ as a substrate for deacetylation. I refer readers to a previous review detailing how Acetyl-CoA levels are dynamically responsive to nutrient availability, affecting histone acetylation and gene expression (Sivanand et al., 2018). Additionally, starvation blocks global protein translation by inhibiting mTOR complex 1 (mTORC1), which in turn limits cellular capabilities for intra and intercellular signaling and gene expression dynamics (Holcik and Sonenberg, 2005; Wullschleger et al., 2006). Recently, it has been further shown that fasting effects on gene transcription and translation depend upon the nature of the specific nutrients that are deprived (Gameiro and Struhl, 2018). Currently, we know almost nothing about how global or specific nutrient deprivations

affect stochastic cellular epigenetic diversity and adaptability, indicating an important area for study.

In a baseline fed state, diverse epigenetic writers, readers and erasers are available with readily available substrates and co-factors. However, after ∼3 days of fasting, the human body transitions into an early starvation state designed to preserve cellular proteins and this involves a major switch to ketone metabolism, which shifts epigenetic regulatory processes and triggers protective stress responses (Dai et al., 2020). It is reasonable to expect that this can help to constrain and inhibit biochemical reactions that promote cellular epigenetic diversity and adaptability, but remains to be tested. New single cell omics technologies, including single cell RNASeq and ATAC-Seq can help test these predictions directly. The implication of identifying interventions that can constrain cellular diversity and adaptability is that we can couple these interventions with chemotherapy strikes and improved drugs to increase chances for a cure and complete disease elimination. If such constraints can be induced, we should expect that the type and duration of nutrient deprivations can be tailored to different cancer types, stages and lesion locations.

## HOW COULD CHANGES TO BODY TEMPERATURE HELP CONSTRAIN CANCER CELL DIVERSITY AND ADAPTABILITY TO FACILITATE TUMOR CELL EXTINCTION?

As described above, chromatin methylation and acetylation biochemical reaction kinetics can be controlled by limiting their substrates and cofactors through nutrient deprivation. However, another important factor constraining these biochemical reactions is body temperature, which controls reaction kinetics (e.g., Kd, dissociation constant). Whole organism metabolic rate scales with the 3/4-power of body mass and increases exponentially with temperature, up to ∼40°C when catabolic processes increase (Gillooly et al., 2001). Body temperature profoundly affects metabolic rates and vice versa. A 1% increase in body temperature has been linked to a 10–15% increase in metabolic rate in endotherms (Evans et al., 2015). On the other hand, decreasing body temperature slows biochemical reaction kinetics. Most physiological processes and biochemical reactions function optimally at ∼37°C (98°F). What temperature state might best help to drive cancer cell extinction?

As noted above, the body responds to microbial infections through a combination of fasting and fever, and the overall sickness response can eliminate cancer in patients. A high-grade fever in an adult is an oral temperature of > 39.4°C (103°F), which can begin to promote catabolic processes and the denaturation of enzymes. In contrast, reducing body temperature slows cellular reaction kinetics and decreases the metabolic rate (Evans et al., 2015). For example, hibernation (torpor) involves a profoundly decreased metabolic rate in order to survive harsh environmental conditions with low nutrient availability (Carey et al., 2003). For torpor to occur, a first step is to trigger a decrease the hypothalamic set point for body temperature, and then in turn drop the body temperature for the induction of torpor and decreased metabolic rate (Gillooly et al., 2001; Geiser, 2004). A tumor in a hibernating animal stops growing during hibernation, but then resumes growth after torpor (Lyman and Fawcett, 1954). Dangerous, transient hypometabolic hibernation-like states can also occur in human cancers and these subpopulations of persistent, dormant cancer cells are major causes of mortality, disease recurrence and treatment failure (Recasens and Munoz, 2019; Shen et al., 2020). Recently, it was shown that most cancer cells have the capability to enter a dormant (or diapause) state (Recasens and Munoz, 2019). Out of the many adaptive phenotypes cancer cell subpopulations might occupy, this hypometabolic state is one of the most dangerous. Previous studies have shown that chronic cold stress, which depresses metabolic rate and immunity, is associated with elevated risks for cancer (Bandyopadhayaya et al., 2020). Cold stress is associated with accelerated tumor growth and treatment resistance in mice, which appears to involve enhanced tumor cell survival pathways as well as suppressed anti-tumor immunity (Kokolus et al., 2013; Messmer et al., 2014). This suggests that increased temperature could help prevent cancer cell dormancy and hibernation-like states in the body (**Figure 6A**).

So far, thinking in the cancer field has primarily focused on uncovering molecular targets that might help block cancer cell dormancy using targeted drugs (Recasens and Munoz, 2019). However, transiently elevating body temperature and metabolic rates might be effective, particularly when coupled with fasting and chemotherapy. Moreover, the activation of the immune system, which occurs in response to elevated body temperature, might beneficially boost anti-tumor immunity (Evans et al., 2015). There is clinical evidence and multiple independent studies showing that hyperthermia has therapeutically beneficial effects in cancer patients (Hildebrandt et al., 2002; van der Zee, 2002; Wust et al., 2002; Jha et al., 2016). Efforts to use hyperthermia clinically have included local delivery of microwaves or radiowaves, as well as approaches to use arrays of antennas to heat entire body parts. For metastatic patients, whole body heating approaches using radiant thermal isolation systems have been developed that can achieve systemic temperatures of 41.8–42.0°C. Perfect thermal isolation is sufficient in-and-of itself to raise the body temperature from 37.5 to 42 in a 70 kg patient in 180 min (Wust et al., 2002). In mice, simply raising the ambient temperature from 22°C to 30°C causes measurable improvements in tumor sensitivity to chemotherapy treatment (Eng et al., 2015). The benefits of hyperthermia are thought to include immune activation and the enhancement of anti-tumor immunity, increased blood perfusion and drug delivery into tumor sites, and cytotoxic effects on tumor cells growing in low pO2 and low pH conditions. Effectiveness for hyperthermia for different cancers has been reported in some randomized clinical trials (Jha et al., 2016).

In addition to beneficial immune activation, perfusion and drug uptake effects, fever/hyperthermia promotes increased cellular metabolic rates and biochemical reaction kinetics, and then at ∼40°C, causes enzyme denaturation and a rapid decline in reaction kinetics. Such effects could be applied precisely to help

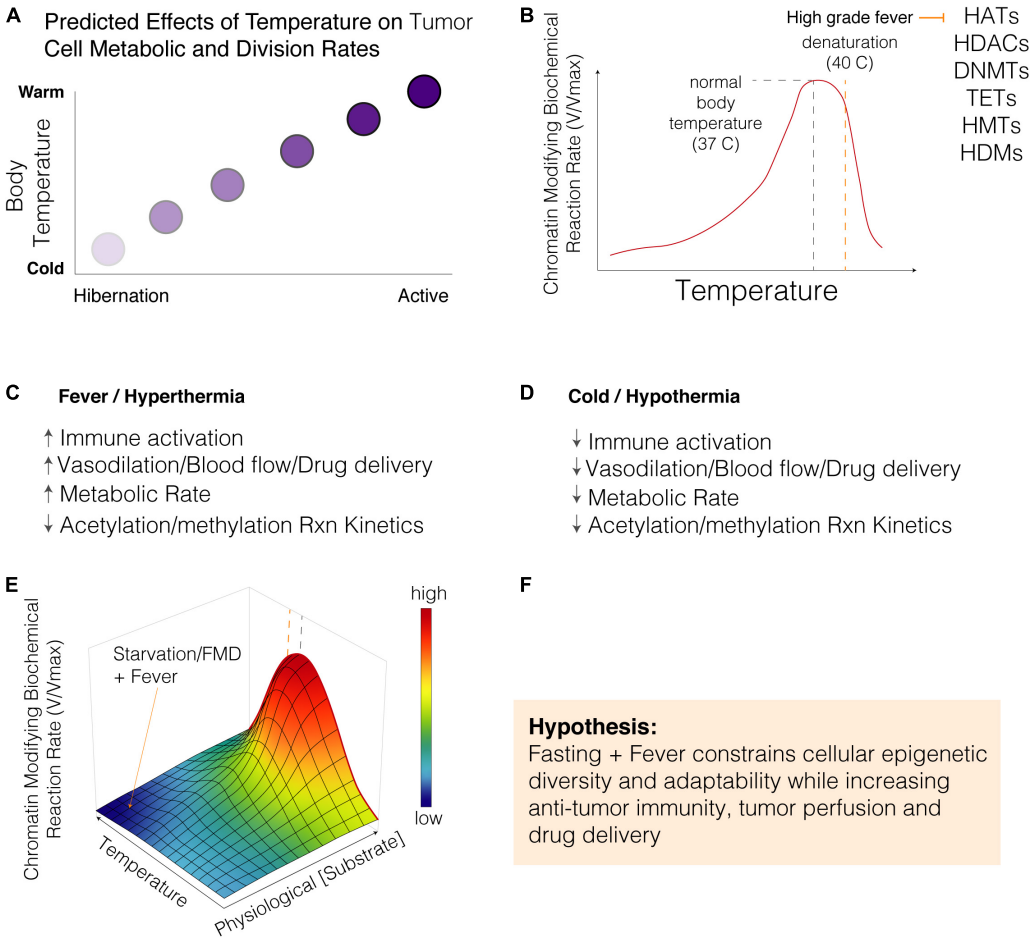


**FIGURE 6 |** Fever/hyperthermia is predicted to block cellular hypometabolic states and constrain epigenetic diversity and adaptability by impairing enzyme activity. **(A)** Cold stress depresses metabolic rate and promotes hypometabolic states, while warming increases metabolic rates. Warming could help block hibernation-like states in cancer cell subpopulations. **(B)** Fever/hyperthermia triggers rapid decreases in biochemical reaction rates due to denaturation of enzyme active sites. Thus, fever-level body temperatures may help inhibit the activity of chromatin-modifying enzymes and block epigenetic diversity and adaptability. **(C,D)** Compare the effects of hyperthermia versus hypothermia and suggest that hyperthermic treatments could have multiple beneficial effects for cancer elimination. **(E)** Plot shows that combining starvation/FMD with fever/hyperthermia could be a potent intervention constraining tumor cell epigenetic diversity and adaptability. **(F)** A hypothesis for the benefits of combining starvation/FMD with fever/hyperthermia.

prevent cancer cells from occupying dangerous hypometabolic states, and at $> 40°C$, to help narrow the dynamic range of biochemical reaction kinetics in cells and thereby the diversity of epigenetic states cancer cells can occupy (**Figures 6B–D**). Overall, high-grade fever/hyperthermia may reduce cancer cell diversity and adaptability. When applied in combination with starvation/FMD, cells are struck with the combination of limited substrates and cofactors plus a forced increase in metabolic rates and enzymatic denaturation (**Figures 6E,F**, 7). In patients, simple approaches to transiently increase body temperature and metabolic rate could be vigorous exercise, which is difficult for patients, or dry sauna treatments (Hussain and Cohen, 2018). Dry saunas begin to raise core body temperature within 15 min (Zalewski et al., 2014). More aggressive approaches could involve pyrogen treatments, like LPS (lipopolysaccharide). Further studies are needed to test whether mimicking the vertebrate sickness response with a fasting + fever combinatorial therapy has the effect of helping to constrain tumor cell epigenetic diversity and adaptability and making cancer cells more vulnerable to chemotherapy treatment strikes.

## TIMING THE STRIKE—CELLULAR SYNCHRONIZATION THROUGH STARVATION AND TEMPERATURE CHANGE CYCLES

For decades, scientists have used nutrient withdrawal or temperature changes to synchronize the proliferation of dividing bacterial, yeast or mammalian cells in culture for experiments. The removal of serum from culture media causes cells to withdraw from the cell cycle (Pardee, 1974; Zetterberg and Larsson, 1985; Balsalobre et al., 1998). Then, the subsequent

re-introduction of serum triggers the cell population to synchronously re-enter the cell cycle, homogenizing the cell population. Repeated serum removal cycles can synchronize the division of 80% of cells in culture (Bánfalvi, 2011; Tian et al., 2012). Similarly, reducing and then increasing the ambient temperature can synchronize dividing cells in culture (Rieder and Cole, 2002; Bánfalvi, 2017). This suggests that cycles of starvation, re-feeding and increased temperature could be combined in patients and timed with chemotherapy treatments to help reduce cellular diversity by synchronizing tumor cell populations for maximum killing during a chemotherapy treatment strike (**Figure 8**). Different schedules might be developed for different phases of treatment for complete elimination of metastatic disease.

For a treatment regimen to have the potential to be curative, it is predicted that the treatment needs to achieve NED (no evidence of disease), thereby eliminating the majority of the disease, and then continue with diverse strikes to ultimately eliminate the remaining, yet undetectable disease (Gatenby et al., 2020). If patients are to be cured, maintaining health for a rich, long life must be integral to the approach and the functionality of the immune and digestive systems should be preserved. Thus, the major initial objectives are to (1) maximize tumor cell killing to reach NED as quickly as possible and with as few drugs as possible, and (2) to reach NED with paradigms that protect the long-term health and quality of life of the patient as much as possible (**Figure 9A**). By reaching NED quickly, safely and efficiently, the tumor cell population is fragmented and vulnerable to extinction with continued treatments (see below). Ideally, the approach involves delivering successive combinations of treatment strikes using different drugs that attack different mechanisms and switching to each new treatment prior to progression. Switching treatments at the disease nadir, but prior to progression potentially helps to preserve drug efficacy for future use if needed.

Toward achieving these goals in actual patients, coupling starvation and hyperthermia cycles with strikes of cytotoxic chemotherapy infusions is expected to enhance tumor cell killing by (1) sensitizing the tumor to treatment, (2) reducing growth signals driving tumor proliferation (e.g., glucose, insulin, IGF1, leptin), (3) impairing capabilities to sustain metabolically demanding drug resistance mechanisms, (4) disrupting the Warburg effect, (5) boosting anti-tumor immunity through hyperthermia, (6) improving vasodilation and drug delivery to tumor cells, and finally, (7) constraining capabilities for cellular epigenetic diversity, adaptability and dormancy during treatment (**Figure 9B**). Moreover, starvation appears to protect normal cells from the cytotoxic effects of chemotherapies by reducing division and metabolic activity, while a majority of tumor cells continue to divide and be affected, resulting in differential sensitivity to chemotherapy treatment (Lee et al., 2012). Thus, integrating starvation and hyperthermia cycles into the first phase of attack and applying the chemotherapy strikes during the starvation response could help the patient reach NED quickly and with fewer drugs and treatment cycles. Moreover, it may help protect the health of the patient during the aggressive treatment or even enable a more aggressive regimen to achieve NED.

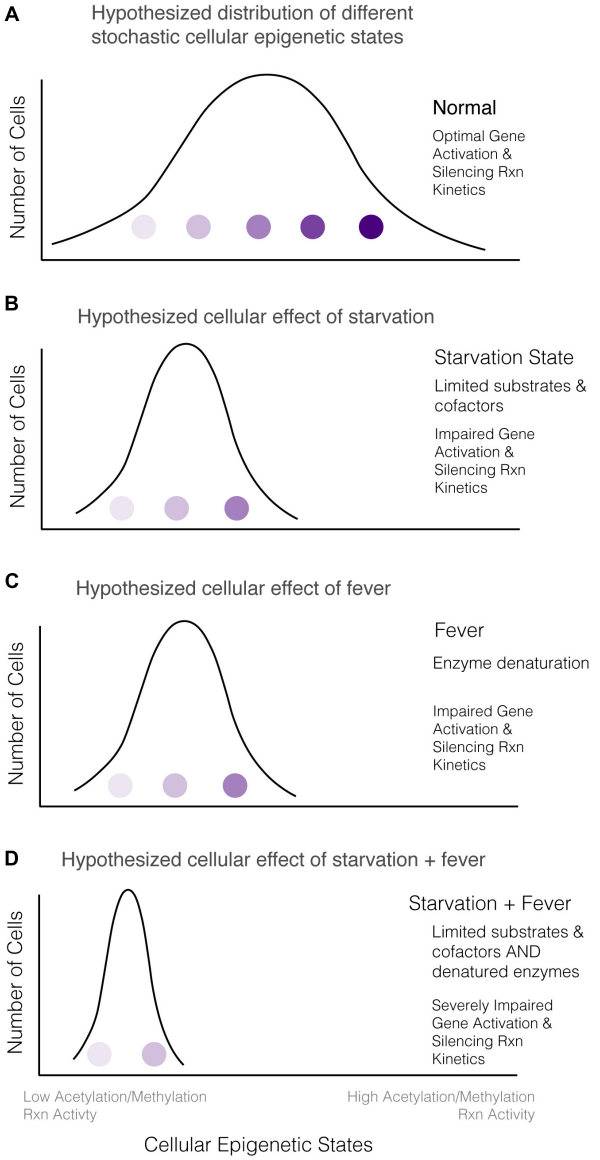


**FIGURE 7 |** Schematic depiction of the hypothesized effects of combining starvation/FMD and fever/hyperthermia on tumor cell epigenetic diversity. **(A)** At baseline, enzyme kinetics are optimal and all possible stochastic epigenetic cellular states can be occupied in a population of cells. **(B)** Starvation limits essential substrates and cofactors for chromatin modifying enzymes, constraining epigenetic dynamics the number of states that a cell can occupy. **(C)** Fever/hyperthermia denatures chromatin modifying enzymes, inhibiting their activity and constraining the number of different epigenetic states that a cell can potentially occupy. **(D)** The combination of starvation and fever/hyperthermia creates a state that severely constrains cancer cell potential for epigenetic diversity.

Once NED is achieved, the next major challenge is to eliminate any persistent, hibernating tumor cells that entered dormancy and have evaded treatment, but will cause disease recurrence in the future (**Figures 9A,C**). During this stage, when the tumor cell population is fragmented and vulnerable, chemotherapy and hyperthermia strikes might be best timed with the re-feeding

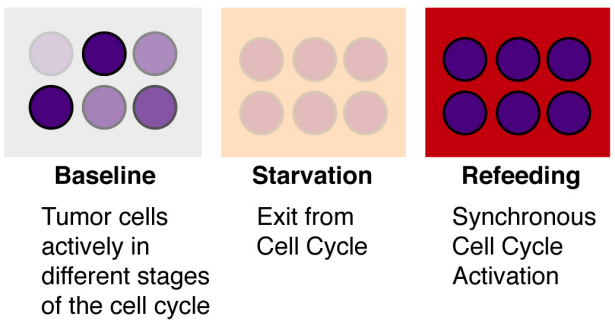


**FIGURE 8 |** Cycles of transient starvation/FMD are predicted to help synchronize tumor cell division and further reduce heterogeneity. At baseline tumor cells are dividing at different rates and are in different stages of the cell cycle. Following starvation, the division of tumor cells that are sensitive to environmental nutrient concentrations is stalled. Many tumor cells are not sensitive to nutrients and continue to divide and are sensitive to chemotherapy. Subpopulations of tumor cells that are sensitive to nutrients, may stop division and enter persistent, hypometabolic states during starvation. However, upon re-feeding, nutrient sensitive tumor cells are expected to synchronously enter the cell cycle, as is observed for some cancer cell lines *in vitro* upon serum-induced cell cycle activation. This activation is expected to make the cell population more homogeneous and vulnerable to chemotherapy.

phase following a starvation cycle to help activate dormant cancer cells and make them sensitive to treatment. Re-feeding after starvation appears to drive progenitor cells, stem cells and tumor cells into a rebound of increased, synchronous cell division (Stragand et al., 1979; Brandhorst et al., 2015), though more studies are needed. As noted above, cells in culture synchronously re-enter the cell cycle upon the return of serum or shift from cold to warm temperature. However, while this approach is predicted to help activate and sensitize persistent, hibernating tumor cells to chemotherapy treatment, it is also expected to increase unwanted damage to healthy cell populations that also enter the cell cycle upon re-feeding. Therefore, this hypothetical approach for eliminating hibernating tumor cells might be best applied in a limited and strategic manner when NED has been reached and there is a chance to now cure the disease with continued aggressive therapy.

Finally, I propose a third and final phase of adjuvant attack during NED that uses remaining drugs in the clinical arsenal that are designed for continuous chronic delivery and have low side effects, such as oral chemotherapies (e.g., Capecitabine) or targeted therapies (e.g., aromatase inhibitors and/or Palbociclib, etc.). Frequently, these would be the first line therapies, but would be delivered chronically until progression. Instead, here they are delivered in this final adjuvant phase to drive disease extinction. The continuous chronic delivery is potentially advantageous for treating slow dividing and dormant tumor cells. Importantly however, this chronic drug treatment is now coupled with randomized and unpredictable starvation, fasting, dietary and hyperthermic metabolic switches (**Figure 9D**). The idea is that by inducing randomized and different metabolic states during treatment, the metabolic, epigenetic and gene expression states of the tumor cells are forced into flux and occupy many different molecular states during the chronic drug treatment. Selection for drug resistance is strongly driven by the application of chronic, *unchanging* treatment conditions and unpredictable environmental changes are primary factor in driving extinction (Gatenby et al., 2019, 2020). Therefore, by enforcing randomized metabolic switching, one aims to (i) force any drug resistant cellular states to be transient and disrupted before a dangerous new resistant cell lineage becomes stable, (ii) drive dormant tumor cells into states of vulnerability to the treatment, and (iii) stochastically "screen" different molecular states in the remaining tumor cell population to uncover those that sensitize different cells to the drug. Randomly perturbing metabolic pathways during chronic treatment over time likely offers one of the best chances of driving tumor cells throughout the body into different molecular states that break evolutionary trajectories and expose drug-sensitive vulnerabilities that ultimately lead to extinction. Devising optimal approaches to do this is an important area for study and we already have an understanding of different dietary and fasting strategies for metabolic switching that the field can start with (Nencioni et al., 2018; Tajan and Vousden, 2020).

Most importantly for clinical applications, there are reasons to believe that this type of program will be tolerable and safe and easily to integrate into the care setting. Pre-clinical studies of FMD in mice involved treatments that are typically chronic in patients, including fulvestrant and palbociclib, and are safe and effective (Caffa et al., 2020). Further, in FMD clinical studies of patients taking drugs during the fasting and re-feeding periods, the approach appears safe (de Groot et al., 2020). Nonetheless, the starvation-to-re-feeding pulse with drug strikes warrants further investigation. The addition of hyperthermia during re-feeding, which will increase perfusion and drug delivery, might be best reserved for drugs with low probabilities of causing neuropathy. The timing of starvation + hyperthermic interventions might also be most effective when timed with the known pharmacokinetics of the drugs to maximize the tumor cell-killing window. For example, palbociclib reaches peak plasma concentrations 6–12 h after oral administration and a steady state in the body after 8 days of treatment, suggesting that starvation, re-feeding and/or hyperthermic interventions are best done 8 days into the typical 21 day treatment cycle and 6–12 h after taking a dose. One question is whether the re-feeding period can be optimized with specific nutrients intended to activate cancer cell populations that especially depend on glucose, amino acid or fatty acid metabolism, or other nutrients. Additionally, supplementing fasting, hyperthermia and chemotherapy strikes with small molecule drugs that impair major metabolic pathways, like inhibitors of heat shock proteins, ubiquitin mediated protein degradation, mTOR signaling or others, may increase the epigenetic homogenization of tumor cells and/or help disrupt epigenetic and gene expression states of drug resistance. These general concepts need to be tested for safety and efficacy in mouse models and tested for associations with effects on tumor cell synchronization and constrained epigenetic diversity using

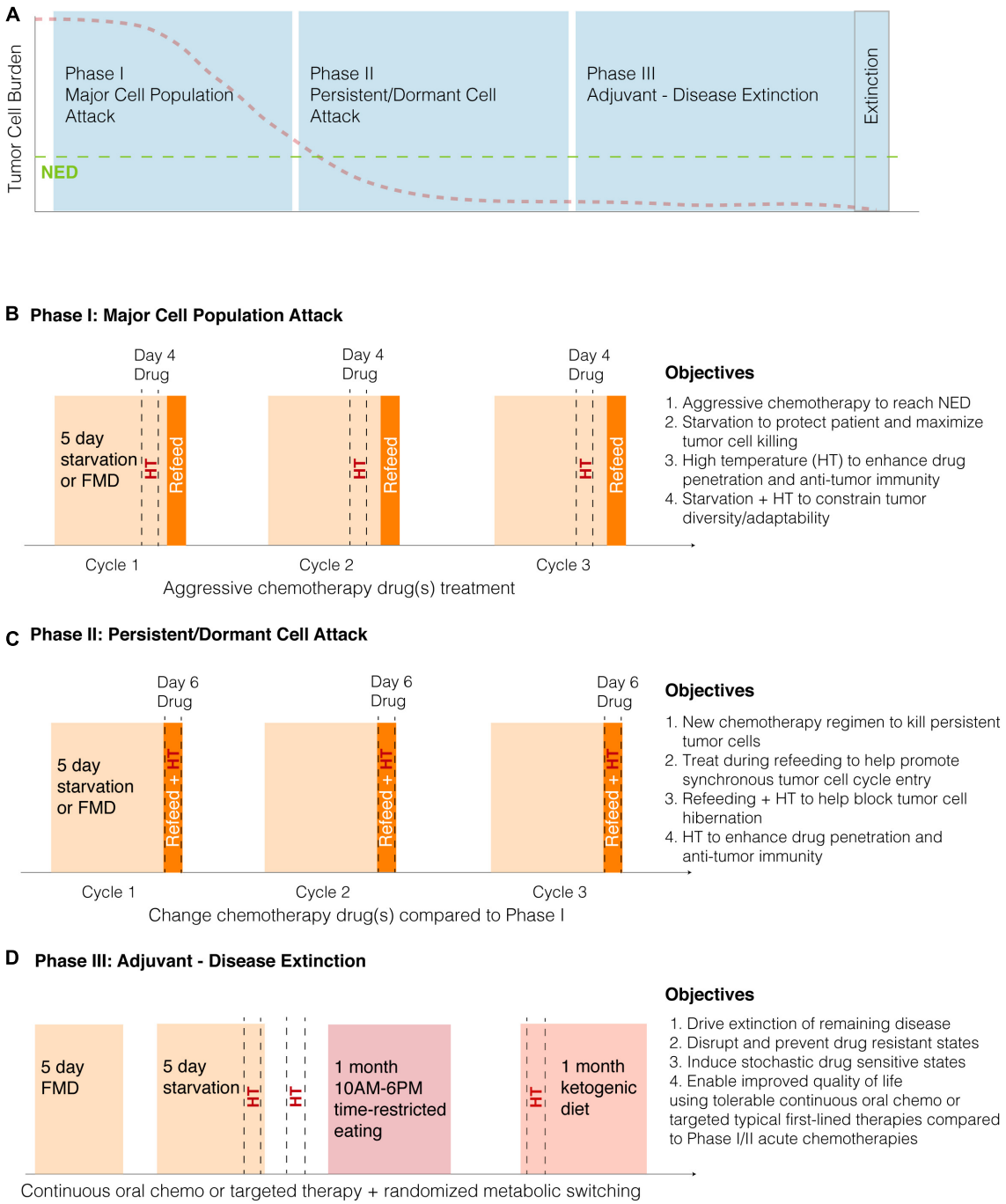


**FIGURE 9 |** A three-phase paradigm for combining starvation/FMD/diet with hyperthermia and drug treatments to promote metastatic disease extinction. **(A)** The schematic depicts two phases of combinatorial treatment to eliminate the disease. In Phase I, the goal is to rapidly drive the disease to NED using combinations of drug treatments, starvation and hyperthermia. In Phase II, the goal is to activate, sensitive and kill persistent and dormant tumor cells in the body that will lead to the recurrence of the disease in the future. In Phase III, the goal is to drive the disease to extinction by combining a continuous drug treatment that enables a high quality of life with randomized starvation, intermittent fasting, dietary and hyperthermic interventions that cause unpredictable and different metabolic states to eliminate remaining disease and prevent recurrence. **(B)** In Phase I, by delivering drug treatment strikes during starvation periods (day 4 of starvation or FMD), tumor cells are sensitized to treatment and healthy cells are protected, improving drug efficacy and protecting patient health and quality of life. By including hyperthermia (HT) during drug treatment strikes, drug penetration into tumor sites may increase, hypometabolic states may be blocked and tumor cell diversity and adaptability are expected to be impaired. **(C)** In Phase II, the drug strikes and HT are applied during the re-feeding period after a starvation/FMD cycle, where the goal is to flush out persistent hypometabolic cancer cells and drive them into cell division, homogenize them and kill them with treatment. **(D)** In Phase III, continuous oral chemo or targeted therapies are used in combination with randomized and unpredictable metabolic switches to disrupt the epigenetic and gene expression cancer cell landscapes to break emerging drug resistant phenotypes and stochastically induce drug sensitive states over time, ultimately leading to complete disease extinction.

recent paired single cell RNASeq and ATAC-Seq technologies. Finally, treating patients that have reached NED by scan and tumor markers leaves the clinician in the dark regarding how to gage the efficacy of such treatments. Circulating tumor DNA might be a more sensitive and effective marker during this phase to help define the bona fide extinction of the disease.

## CONCLUSION

Stochastic epigenetic and gene expression states are primary drivers of the evolution of drug resistance in patients. Moreover, the diploid/polyploid genome of tumor cells likely enables profound epigenetic, allelic and gene expression diversity at the cellular level through stochastic DAE and clonal RME. These gene and allele level epigenetic and gene expression effects are barriers to the elimination of metastatic disease. To create clinical solutions, we need an understanding of the short-term and long-term epigenetic mechanisms involved, their regulation by different metabolic mechanisms and their roles in the initiation and persistence of cancer and the evolution of drug resistance. No single drug will solve the cancer evolution problem. Here, I have attempted to draw on the fields of evolution, ecology, infectious disease, epigenetics, metabolism, biochemistry, genomics and oncology to synthesize a clinically feasible path forward to help homogenize tumor cell populations and constrain cellular epigenetic diversity and adaptability in patients. The rationale for pursuing this objective is to improve the efficacy of combinatorial chemotherapy strikes to cure metastatic disease. I propose an approach that involves combining cycles of starvation and hyperthermia to synchronize tumor cell division and constrain tumor cell epigenetic diversity and adaptability by transiently limiting essential nutrients, substrates and cofactors and impairing the optimal enzymatic activity of chromatin modifying enzymes when drug treatment strikes are delivered. I then propose inducing unpredictable different metabolic states during continuous oral drug treatments to drive the disease to extinction. In addition, I speculate that strategic increases in body temperature may help block cancer cells from entering dangerous hypometabolic, hibernation-like states during treatment, which are major barriers to disease elimination. Whether these manipulations can actually reduce tumor cell epigenetic diversity, stochastic variability and adaptability and improve outcomes is not known and remains to be tested using state-of-the-art single cell genomics methods and pre-clinical studies. Nonetheless, based on simple biochemical principles, it is reasonable to expect that carefully timed, transient starvation and hyperthermic cycles will limit the range of molecular states tumor cells can occupy during chemotherapy infusion treatments. Further, given the evidence that drug resistance is a stochastic and dynamic molecular state that cells are able to move in and out of, rather than a genetically hardwired state, it is also reasonable to expect that unpredictable switches of metabolic states over time during continuous treatment can help to disrupt drug resistant epigenetic and gene expression states and induce drug sensitive states in tumor cells. This especially important when the available drugs in the arsenal are limited.

In summary, to achieve cancer cell extinction, I propose a three-phase paradigm. In Phase I, starvation and hyperthermia cycles are timed with chemotherapy strikes in a manner that is anticipated to protect the patient and enable aggressive treatments that help to rapidly drive metastatic disease to NED. In Phase II, chemotherapy treatment strikes and hyperthermia are timed with the re-feeding phase after a starvation cycle, where the goal is to help activate, sensitize and eliminate persistent, hibernating cancer cells and help completely wipe out the disease. In Phase III, continuous chemo or targeted therapies that enable improved quality of life are combined with randomized and diverse metabolic state switches using different starvation, fasting, dietary and hyperthermia treatments to drive extinction. This paradigm integrates knowledge from different fields, including the ability of the vertebrate infection response to eliminate cancer. There are many unknowns and opportunities for study in this area to help patients.

## AUTHOR CONTRIBUTIONS

CG conceived and wrote the article.

## FUNDING

This work was supported by grants to CG from the National Institutes of Health, including R01MH109577, R01AG064013, R21AG067350, R21MH118570, and R21MH120468.

## ACKNOWLEDGMENTS

I thank Elliott Ferris and Stephanie Kravitz in the Gregg Lab, and Bob Gatenby, Alana Welm, and Saundra Buys for reading and commenting on this article. I thank Joel Brown, Dawn Lemanne, and Valter Longo for helpful discussions.

## REFERENCES

Adelman, J. S., and Martin, L. B. (2009). Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. *Integr. Comp. Biol.* 49, 202–214. doi: 10.1093/icb/icp028

Aubert, A. (1999). Sickness and behaviour in animals: a motivational perspective. *Neurosci. Biobehav. Rev.* 23, 1029–1036. doi: 10.1016/s0149-7634(99)00034-2

Balázsi, G., van Oudenaarden, A., and Collins, J. J. (2011). Cellular decision making and biological noise: from microbes to mammals. *Cell* 144, 910–925. doi: 10.1016/j.cell.2011.01.030

Balsalobre, A., Damiola, F., and Schibler, U. (1998). A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* 93, 929–937. doi: 10.1016/s0092-8674(00)81199-x

Bandyopadhayaya, S., Ford, B., and Mandal, C. C. (2020). Cold-hearted: A case for cold stress in cancer risk. *J. Therm. Biol.* 91:102608. doi: 10.1016/j.jtherbio.2020.102608

Bánfalvi, G. (2011). *Cell Cycle Synchronization : Methods and Protocols*. New York NY: Humana.

Bánfalvi, G. (2017). Overview of cell synchronization. *Methods Mol. Biol.* 1524, 3–27. doi: 10.1007/978-1-4939-6603-5_1

Baylin, S. B., and Jones, P. A. (2016). Epigenetic determinants of cancer. *Cold Spring Harb. Perspect. Biol.* 8:a019505. doi: 10.1101/cshperspect.a019505

Bennett, R. L., and Licht, J. D. (2018). Targeting epigenetics in cancer. *Annu. Rev. Pharmacol. Toxicol.* 58, 187–207. doi: 10.1146/annurev-pharmtox-010716-105106

Bintu, L., Yong, J., Antebi, Y. E., McCue, K., Kazuki, Y., Uno, N., et al. (2016). Dynamics of epigenetic regulation at the single-cell level. *Science* 351, 720–724. doi: 10.1126/science.aab2956

Blake, W. J., Kærn, M., Cantor, C. R., and Collins, J. J. (2003). Noise in eukaryotic gene expression. *Nature* 422, 633–637. doi: 10.1038/nature01546

Branciamore, S., Valo, Z., Li, M., Wang, J., Riggs, A. D., and Singer-Sam, J. (2018). Frequent monoallelic or skewed expression for developmental genes in CNS-derived cells and evidence for balancing selection. *Proc. Natl. Acad. Sci. U.S.A.* 115, E10379–E10386. doi: 10.1073/pnas.1808652115

Brandhorst, S., Choi, I. Y., Wei, M., Cheng, C.-W., Sedrakyan, S., Navarrete, G., et al. (2015). A Periodic diet that mimics fasting promotes multi-system regeneration, enhanced cognitive performance, and healthspan. *Cell Metab.* 22, 86–99. doi: 10.1016/j.cmet.2015.05.012

Caffa, I., Spagnolo, V., Vernieri, C., Valdemarin, F., Becherini, P., Wei, M., et al. (2020). Fasting-mimicking diet and hormone therapy induce breast cancer regression. *Nature* 583, 620–624. doi: 10.1038/s41586-020-2502-7

Campbell, S. L., and Wellen, K. E. (2018). Metabolic signaling to the nucleus in cancer. *Mol. Cell* 71, 398–408. doi: 10.1016/j.molcel.2018.07.015

Carey, H. V., Andrews, M. T., and Martin, S. L. (2003). Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol. Rev.* 83, 1153–1181. doi: 10.1152/physrev.00008.2003

Carrer, A., Parris, J. L. D., Trefely, S., Henry, R. A., Montgomery, D. C., Torres, A., et al. (2017). Impact of a high-fat diet on tissue acyl-coa and histone acetylation levels. *J. Biol. Chem.* 292, 3312–3322. doi: 10.1074/jbc.M116.750620

Carroll, S. B. (2008). Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* 134, 25–36. doi: 10.1016/j.cell.2008.06.030

Chandradoss, K. R., Chawla, B., Dhuppar, S., Nayak, R., Ramachandran, R., Kurukuti, S., et al. (2020). CTCF-mediated genome architecture regulates the dosage of mitotically stable mono-allelic expression of autosomal genes. *Cell Rep.* 33:108302. doi: 10.1016/j.celrep.2020.108302

Cheng, Y., He, C., Wang, M., Ma, X., Mo, F., Yang, S., et al. (2019). Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Trans. Target. Ther.* 4:62. doi: 10.1038/s41392-019-0095-0

Choudhary, C., Weinert, B. T., Nishida, Y., Verdin, E., and Mann, M. (2014). The growing landscape of lysine acetylation links metabolism and cell signalling. *Nat. Rev. Mol. Cell Biol.* 15, 536–550. doi: 10.1038/nrm3841

Cluntun, A. A., Huang, H., Dai, L., Liu, X., Zhao, Y., and Locasale, J. W. (2015). The rate of glycolysis quantitatively mediates specific histone acetylation sites. *Cancer Metab.* 3:10. doi: 10.1186/s40170-015-0135-3

Coward, J., and Harding, A. (2014). Size does matter: why polyploid tumor cells are critical drug targets in the war on cancer. *Front. Oncol.* 4:123. doi: 10.3389/fonc.2014.00123

Dai, Z., Ramesh, V., and Locasale, J. W. (2020). The evolving metabolic landscape of chromatin biology and epigenetics. *Nat. Rev. Genet.* 21, 737–753. doi: 10.1038/s41576-020-0270-8

Dantzer, R., and Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain Behav. Immun.* 21, 153–160. doi: 10.1016/j.bbi.2006.09.006

Davidson, E. H. (2010). Emerging properties of animal gene regulatory networks. *Nature* 468, 911–920. doi: 10.1038/nature09645

Davidson, E. H., and Erwin, D. H. (2006). Gene regulatory networks and the evolution of animal body plans. *Science* 311, 796–800. doi: 10.1126/science.1113832

de Cabo, R., and Mattson, M. P. (2019). Effects of intermittent fasting on health, aging, and disease. *N. Engl. J. Med.* 381, 2541–2551. doi: 10.1056/NEJMra1905136

de Groot, S., Lugtenberg, R. T., Cohen, D., Welters, M. J. P., Ehsan, I., Vreeswijk, M. P. G., et al. (2020). Fasting mimicking diet as an adjunct to neoadjuvant chemotherapy for breast cancer in the multicentre randomized phase 2 DIRECT trial. *Nat. Commun.* 11:3083. doi: 10.1038/s41467-020-16138-3

Del Monte, U. (2009). Does the cell number 10(9) still really fit one gram of tumor tissue? *Cell Cycle* 8, 505–506. doi: 10.4161/cc.8.3.7608

Deng, Q., Ramskold, D., Reinius, B., and Sandberg, R. (2014). Single-cell RNA-seq reveals dynamic, random monoallelic gene expression in mammalian cells. *Science* 343, 193–196. doi: 10.1126/science.1245316

Dobosz, P., and Dzieciątkowski, T. (2019). The Intriguing History of Cancer Immunotherapy. *Front. Immunol.* 10:2965. doi: 10.3389/fimmu.2019.02965

Easwaran, H., Tsai, H.-C., and Baylin, S. B. (2014). Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. *Mol. Cell* 54, 716–727. doi: 10.1016/j.molcel.2014.05.015

Eckersley-Maslin, M. A., Thybert, D., Bergmann, J. H., Marioni, J. C., Flicek, P., and Spector, D. L. (2014). Random monoallelic gene expression increases upon embryonic stem cell differentiation. *Dev. Cell* 28, 351–365. doi: 10.1016/j.devcel.2014.01.017

Eng, J. W. L., Reed, C. B., Kokolus, K. M., Pitoniak, R., Utley, A., Bucsek, M. J., et al. (2015). Housing temperature-induced stress drives therapeutic resistance in murine tumour models through β2-adrenergic receptor activation. *Nat. Commun.* 6:6426. doi: 10.1038/ncomms7426

Evans, S. S., Repasky, E. A., and Fisher, D. T. (2015). Fever and the thermal regulation of immunity: the immune system feels the heat. *Nat. Rev. Immunol.* 15, 335–349. doi: 10.1038/nri3843

Feinberg, A. P., and Irizarry, R. A. (2010). Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. *Proc. Natl. Acad. Sc.i U.S.A.* 107, 1757–1764. doi: 10.1073/pnas.0906183107

Flavahan, W. A., Gaskell, E., and Bernstein, B. E. (2017). Epigenetic plasticity and the hallmarks of cancer. *Science* 357:eaal2380. doi: 10.1126/science.aal2380

Fox, D. T., Soltis, D. E., Soltis, P. S., Ashman, T.-L., and Van de Peer, Y. (2020). Polyploidy: a biological force from cells to ecosystems. *Trends Cell Biol.* 30, 688–694. doi: 10.1016/j.tcb.2020.06.006

Gaiti, F., Chaligne, R., Gu, H., Brand, R. M., Kothen-Hill, S., Schulman, R. C., et al. (2019). Epigenetic evolution and lineage histories of chronic lymphocytic leukaemia. *Nature* 569, 576–580. doi: 10.1038/s41586-019-1198-z

Gameiro, P. A., and Struhl, K. (2018). Nutrient deprivation elicits a transcriptional and translational inflammatory response coupled to decreased protein synthesis. *Cell Rep.* 24, 1415–1424. doi: 10.1016/j.celrep.2018.07.021

Gao, X., Sanderson, S. M., Dai, Z., Reid, M. A., Cooper, D. E., Lu, M., et al. (2019). Dietary methionine influences therapy in mouse cancer models and alters human metabolism. *Nature* 572, 397–401. doi: 10.1038/s41586-019-1437-3

Gatenby, R., and Brown, J. (2018). The evolution and ecology of resistance in cancer therapy. *Cold Spring Harb. Perspect. Med.* 8:a033415. doi: 10.1101/cshperspect.a033415

Gatenby, R. A., Artzy-Randrup, Y., Epstein, T., Reed, D. R., and Brown, J. S. (2020). Eradicating metastatic cancer and the eco-evolutionary dynamics of anthropocene extinctions. *Cancer Res.* 80, 613–623. doi: 10.1158/0008-5472.CAN-19-1941

Gatenby, R. A., Zhang, J., and Brown, J. S. (2019). First strike-second strike strategies in metastatic cancer: lessons from the evolutionary dynamics of extinction. *Cancer Res.* 79, 3174–3177. doi: 10.1158/0008-5472.CAN-19-0807

Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu. Rev. Physiol.* 66, 239–274. doi: 10.1146/annurev.physiol.66.032102.115105

Gendrel, A.-V., Attia, M., Chen, C.-J., Diabangouaya, P., Servant, N., Barillot, E., et al. (2014). Developmental dynamics and disease potential of random monoallelic gene expression. *Dev. Cell* 28, 366–380. doi: 10.1016/j.devcel.2014.01.016

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* 293, 2248–2251. doi: 10.1126/science.1061967

Gimelbrant, A., Hutchinson, J. N., Thompson, B. R., and Chess, A. (2007). Widespread monoallelic expression on human autosomes. *Science* 318, 1136–1140. doi: 10.1126/science.1148910

Guo, M., Peng, Y., Gao, A., Du, C., and Herman, J. G. (2019). Epigenetic heterogeneity in cancer. *Biomark Res* 7:23. doi: 10.1186/s40364-019-0174-y

Hart, B. L. (1988). Biological basis of the behavior of sick animals. *Neurosci. Biobehav. Rev.* 12, 123–137. doi: 10.1016/s0149-7634(88)80004-6

Herzig, S., and Shaw, R. J. (2017). AMPK: guardian of metabolism and mitochondrial homeostasis. *Cell Res.* 19, 121–135. doi: 10.1038/nrm.2017.95

Hildebrandt, B., Wust, P., Ahlers, O., Dieing, A., Sreenivasa, G., Kerner, T., et al. (2002). The cellular and molecular basis of hyperthermia. *Critic. Rev. Oncol. Hematol.* 43, 33–56. doi: 10.1016/s1040-8428(01)00179-2

Hinohara, K., Wu, H.-J., Vigneau, S., McDonald, T. O., Igarashi, K. J., Yamamoto, K. N., et al. (2018). KDM5 histone demethylase activity links cellular transcriptomic heterogeneity to therapeutic resistance. *Cancer Cell* 34, 939–953.e9. doi: 10.1016/j.ccell.2018.10.014

Hogg, S. J., Beavis, P. A., Dawson, M. A., and Johnstone, R. W. (2020). Targeting the epigenetic regulation of antitumour immunity. *Nat. Rev. Drug Discov.* 19, 776–800. doi: 10.1038/s41573-020-0077-5

Holcik, M., and Sonenberg, N. (2005). Translational control in stress and apoptosis. *Nat. Rev. Mol. Cell Biol.* 6, 318–327. doi: 10.1038/nrm1618

Huang, S. (2011). On the intrinsic inevitability of cancer: from foetal to fatal attraction. *Semin. Cancer Biol.* 21, 183–199. doi: 10.1016/j.semcancer.2011.05.003

Huang, S. (2012). Tumor progression: chance and necessity in darwinian and lamarckian somatic (mutationless) evolution. *Prog. Biophys. Mol. Biol.* 110, 69–86. doi: 10.1016/j.pbiomolbio.2012.05.001

Huang, S. (2013). Genetic and non-genetic instability in tumor progression: link between the fitness landscape and the epigenetic landscape of cancer cells. *Cancer Metastasis Rev.* 32, 423–448. doi: 10.1007/s10555-013-9435-7

Huang, S. (2021). Reconciling non-genetic plasticity with somatic evolution in cancer. *Trends Cancer* 7, 309–322. doi: 10.1016/j.trecan.2020.12.007

Huang, S., Ernberg, I., and Kauffman, S. (2009). Cancer attractors: a systems view of tumors from a gene network dynamics and developmental perspective. *Semin. Cell Dev. Biol.* 20, 869–876. doi: 10.1016/j.semcdb.2009.07.003

Huang, W.-C., Bennett, K., and Gregg, C. (2018). Epigenetic and cellular diversity in the brain through allele-specific effects. *Trends Neurosci.* 41, 925–937. doi: 10.1016/j.tins.2018.07.005

Huang, W.-C., Ferris, E., Cheng, T., Hörndli, C. S., Gleason, K., Tamminga, C., et al. (2017). Diverse non-genetic, allele-specific expression effects shape genetic architecture at the cellular level in the mammalian brain. *Neuron* 93, 1094–1109.e7. doi: 10.1016/j.neuron.2017.01.033

Hussain, J., and Cohen, M. (2018). Clinical effects of regular dry sauna bathing: a systematic review. *Evid. Based Complement. Alternat. Med.* 2018:1857413. doi: 10.1155/2018/1857413

Jeffries, A. R., Uwanogho, D. A., Cocks, G., Perfect, L. W., Dempster, E., Mill, J., et al. (2016). Erasure and reestablishment of random allelic expression imbalance after epigenetic reprogramming. *RNA* 22, 1620–1630. doi: 10.1261/rna.058347.116

Jenkinson, G., Pujadas, E., Goutsias, J., and Feinberg, A. P. (2017). Potential energy landscapes identify the information- theoretic nature of the epigenome. *Nat. Genet.* 49, 719–729. doi: 10.1038/ng.3811

Jha, S., Sharma, P. K., and Malviya, R. (2016). Hyperthermia: role and risk factor for cancer treatment. *ALS* 10, 161–167. doi: 10.1016/j.als.2016.11.004

Kærn, M., Elston, T. C., Blake, W. J., and Collins, J. J. (2005). Stochasticity in gene expression: from theories to phenotypes. *Nat. Rev. Genet.* 6, 451–464. doi: 10.1038/nrg1615

Katan-Khaykovich, Y., and Struhl, K. (2002). Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal histone acetylation status upon removal of activators and repressors. *Genes. Dev.* 16, 743–752. doi: 10.1101/gad.967302

Knoechel, B., Roderick, J. E., Williamson, K. E., Zhu, J., Lohr, J. G., Cotton, M. J., et al. (2014). An epigenetic mechanism of resistance to targeted therapy in T cell acute lymphoblastic leukemia. *Nat. Genet.* 46, 364–370. doi: 10.1038/ng.2913

Kokolus, K. M., Capitano, M. L., Lee, C.-T., Eng, J. W. L., Waight, J. D., Hylander, B. L., et al. (2013). Baseline tumor growth and immune control in laboratory mice are significantly influenced by subthermoneutral housing temperature. *Proc. Natl. Acad. Sci. U.S.A.* 110, 20176–20181. doi: 10.1073/pnas.1304291110

Krajcovic, M., and Overholtzer, M. (2012). Mechanisms of ploidy increase in human cancers: a new role for cell cannibalism. *Cancer Res.* 72, 1596–1601. doi: 10.1158/0008-5472.CAN-11-3127

Kravitz, S. N., and Gregg, C. (2019). New subtypes of allele-specific epigenetic effects: implications for brain development, function and disease. *Curr. Opin. Neurobiol.* 59, 69–78. doi: 10.1016/j.conb.2019.04.012

Landau, D. A., Clement, K., Ziller, M. J., Boyle, P., Fan, J., Gu, H., et al. (2014). Locally disordered methylation forms the basis of intratumor methylome variation in chronic lymphocytic leukemia. *Cancer Cell* 26, 813–825. doi: 10.1016/j.ccell.2014.10.012

Larsson, A. J. M., Johnsson, P., Hagemann-Jensen, M., Hartmanis, L., Faridani, O. R., Reinius, B., et al. (2018). Genomic encoding of transcriptional burst kinetics. *Nature* 565, 251–254. doi: 10.1038/s41586-018-0836-1

Lee, C., Raffaghello, L., Brandhorst, S., Safdie, F. M., Bianchi, G., Martin-Montalvo, A., et al. (2012). Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. *Sci. Transl. Med.* 4:124ra27. doi: 10.1126/scitranslmed.3003293

Lee, J. V., Carrer, A., Shah, S., Snyder, N. W., Wei, S., Venneti, S., et al. (2014). Akt-dependent metabolic reprogramming regulates tumor cell histone acetylation. *Cell Metab.* 20, 306–319. doi: 10.1016/j.cmet.2014.06.004

Levine, J. H., Lin, Y., and Elowitz, M. B. (2013). Functional roles of pulsing in genetic circuits. *Science* 342, 1193–1200. doi: 10.1126/science.1239999

Li, X. (2002). Tissue-specific regulation of retinal and pituitary precursor cell proliferation. *Science* 297, 1180–1183. doi: 10.1126/science.1073263

Liau, B. B., Sievers, C., Donohue, L. K., Gillespie, S. M., Flavahan, W. A., Miller, T. E., et al. (2017). Adaptive chromatin remodeling drives glioblastoma stem cell plasticity and drug tolerance. *Stem Cell* 20, 233–246.e7. doi: 10.1016/j.stem.2016.11.003

Locasale, J. W., and Cantley, L. C. (2011). Metabolic flux and the regulation of mammalian cell growth. *Cell Metab.* 14, 443–451. doi: 10.1016/j.cmet.2011.07.014

Lomvardas, S., and Maniatis, T. (2016). Histone and DNA modifications as regulators of neuronal development and function. *Cold Spring Harb. Perspect. Biol.* 8:a024208. doi: 10.1101/cshperspect.a024208

Lyman, C. P., and Fawcett, D. W. (1954). The effect of hibernation on the growth of sarcoma in the hamster. *Cancer Res.* 14, 25–28.

Madlung, A. (2012). Polyploidy and its effect on evolutionary success: old questions revisited with new tools. *Heredity* 110, 99–104. doi: 10.1038/hdy.2012.79

Maley, C. C., Aktipis, A., Graham, T. A., Sottoriva, A., Boddy, A. M., Janiszewska, M., et al. (2017). Classifying the evolutionary and ecological features of neoplasms. *Nat. Rev. Cancer* 17, 605–619. doi: 10.1038/nrc.2017.69

Mariño, G., Pietrocola, F., Eisenberg, T., Kong, Y., Malik, S. A., Andryushkova, A., et al. (2014). Regulation of autophagy by cytosolic acetyl-coenzyme A. *Mol. Cell* 53, 710–725. doi: 10.1016/j.molcel.2014.01.016

Marusyk, A., Janiszewska, M., and Polyak, K. (2020). Intratumor heterogeneity: the rosetta stone of therapy resistance. *Cancer Cell* 37, 471–484. doi: 10.1016/j.ccell.2020.03.007

McGranahan, N., Favero, F., de Bruin, E. C., Birkbak, N. J., Szallasi, Z., and Swanton, C. (2015). Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. *Sci. Transl. Med.* 7:283ra54. doi: 10.1126/scitranslmed.aaa1408

McGranahan, N., and Swanton, C. (2017). Clonal heterogeneity and tumor evolution: past. present, and the future. *Cell* 168, 613–628. doi: 10.1016/j.cell.2017.01.018

Mentch, S. J., Mehrmohamadi, M., Huang, L., Liu, X., Gupta, D., Mattocks, D., et al. (2015). Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. *Cell Metab.* 22, 861–873. doi: 10.1016/j.cmet.2015.08.024

Merlo, L. M. F., Pepper, J. W., Reid, B. J., and Maley, C. C. (2006). Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer* 6, 924–935. doi: 10.1038/nrc2013

Messmer, M. N., Kokolus, K. M., Eng, J. W. L., Abrams, S. I., and Repasky, E. A. (2014). Mild cold-stress depresses immune responses: implications for cancer models involving laboratory mice. *Bioessays* 36, 884–891. doi: 10.1002/bies.201400066

Miyanari, Y., and Torres-Padilla, M.-E. (2013). Control of ground-state pluripotency by allelic regulation of Nanog. *Nature* 483, 470–473. doi: 10.1038/nature10807

Monahan, K., Horta, A., and Lomvardas, S. (2019). LHX2- and LDB1-mediated trans interactions regulate olfactory receptor choice. *Nature* 565, 448–453. doi: 10.1038/s41586-018-0845-0

Nencioni, A., Caffa, I., Cortellino, S., and Longo, V. D. (2018). Fasting and cancer: molecular mechanisms and clinical application. *Nat. Rev. Cancer* 18, 707–719. doi: 10.1038/s41568-018-0061-0

Ng, K. K., Yui, M. A., Mehta, A., Siu, S., Irwin, B., Pease, S., et al. (2018). A stochastic epigenetic switch controls the dynamics of T-cell lineage commitment. *Elife* 7:e37851. doi: 10.7554/eLife.37851

Onuchic, V., Lurie, E., Carrero, I., Pawliczek, P., Patel, R. Y., Rozowsky, J., et al. (2018). Allele-specific epigenome maps reveal sequence-dependent stochastic switching at regulatory loci. *Science* 361:eaar3146. doi: 10.1126/science.aar3146

Orr, H. A. (1995). Somatic mutation favors the evolution of diploidy. *Genetics* 139, 1441–1447.

Ozbudak, E. M., Thattai, M., Kurtser, I., Grossman, A. D., and van Oudenaarden, A. (2002). Regulation of noise in the expression of a single gene. *Nat. Genet.* 31, 69–73. doi: 10.1038/ng869

Pardee, A. B. (1974). A restriction point for control of normal animal cell proliferation. *Proc. Natl. Acad. Sci. U.S.A.* 71, 1286–1290. doi: 10.1073/pnas.71.4.1286

Pastore, A., Gaiti, F., Lu, S. X., Brand, R. M., Kulm, S., Chaligne, R., et al. (2019). Corrupted coordination of epigenetic modifications leads to diverging chromatin states and transcriptional heterogeneity in CLL. *Nat. Commun.* 10:1874. doi: 10.1038/s41467-019-09645-5

Pisco, A. O., Brock, A., Zhou, J., Moor, A., Mojtahedi, M., Jackson, D., et al. (2013). Non-darwinian dynamics in therapy-induced cancer drug resistance. *Nat. Commun.* 4:2467. doi: 10.1038/ncomms3467

Pujadas, E., and Feinberg, A. P. (2012). Regulated noise in the epigenetic landscape of development and disease. *Cell* 148, 1123–1131. doi: 10.1016/j.cell.2012.02.045

Raj, A., Rifkin, S. A., Andersen, E., and van Oudenaarden, A. (2010). Variability in gene expression underlies incomplete penetrance. *Nature* 463, 913–918. doi: 10.1038/nature08781

Raj, A., and van Oudenaarden, A. (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* 135, 216–226. doi: 10.1016/j.cell.2008.09.050

Rajagopal, N., Ernst, J., Ray, P., Wu, J., Zhang, M., Kellis, M., et al. (2014). Distinct and predictive histone lysine acetylation patterns at promoters, enhancers, and gene bodies. *G3 (Bethesda)* 4, 2051–2063. doi: 10.1534/g3.114.013565

Recasens, A., and Munoz, L. (2019). Targeting cancer cell dormancy. *Trends Pharmacol. Sci.* 40, 128–141. doi: 10.1016/j.tips.2018.12.004

Reed, D. R., Metts, J., Pressley, M., Fridley, B. L., Hayashi, M., Isakoff, M. S., et al. (2020). An evolutionary framework for treating pediatric sarcomas. *Cancer* 126, 2577–2587. doi: 10.1002/cncr.32777

Reid, M. A., Dai, Z., and Locasale, J. W. (2017). The impact of cellular metabolism on chromatin dynamics and epigenetics. *Cell Res.* 19, 1298–1306. doi: 10.1038/ncb3629

Reinius, B., Mold, J. E., Ramsköld, D., Deng, Q., Johnsson, P., Michaëlsson, J., et al. (2016). Analysis of allelic expression patterns in clonal somatic cells by single-cell RNA-seq. *Nat. Genet.* 48, 1430–1435. doi: 10.1038/ng.3678

Reinius, B., and Sandberg, R. (2015). Random monoallelic expression of autosomal genes: stochastic transcription and allele-level regulation. *Nat. Rev. Genet.* 16, 653–664. doi: 10.1038/nrg3888

Reiter, J. G., Baretti, M., Gerold, J. M., Makohon-Moore, A. P., Daud, A., Iacobuzio-Donahue, C. A., et al. (2019). An analysis of genetic heterogeneity in untreated cancers. *Nat. Rev. Cancer* 19, 639–650. doi: 10.1038/s41568-019-0185-x

Reiter, J. G., Makohon-Moore, A. P., Gerold, J. M., Heyde, A., Attiyeh, M. A., Kohutek, Z. A., et al. (2018). Minimal functional driver gene heterogeneity among untreated metastases. *Science* 361, 1033–1037. doi: 10.1126/science.aat7171

Rieder, C. L., and Cole, R. (2002). Cold-shock and the mammalian cell cycle. *Cell Cycle* 1, 168–174. doi: 10.4161/cc.1.3.119

Risom, T., Langer, E. M., Chapman, M. P., Rantala, J., Fields, A. J., Boniface, C., et al. (2018). Differentiation-state plasticity is a targetable resistance mechanism in basal-like breast cancer. *Nat. Commun.* 9:3815. doi: 10.1038/s41467-018-05729-w

Rv, P., Sundaresh, A., Karunyaa, M., Arun, A., and Gayen, S. (2021). Autosomal Clonal Monoallelic Expression: natural or Artifactual? *Trends Genet* 37, 206–211. doi: 10.1016/j.tig.2020.10.011

Shaffer, S. M., Dunagin, M. C., Torborg, S. R., Torre, E. A., Emert, B., Krepler, C., et al. (2017). Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. *Nature* 546, 431–435. doi: 10.1038/nature22794

Sharma, S. V., Lee, D. Y., Li, B., Quinlan, M. P., Takahashi, F., Maheswaran, S., et al. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* 141, 69–80. doi: 10.1016/j.cell.2010.02.027

Shen, S., Vagner, S., and Robert, C. (2020). Persistent cancer cells: the deadly survivors. *Cell* 183, 860–874. doi: 10.1016/j.cell.2020.10.027

Singer, Z. S., Yong, J., Tischler, J., Hackett, J. A., Altinok, A., Surani, M. A., et al. (2014). Dynamic heterogeneity and dna methylation in embryonic stem cells. *Mol. Cell* 55, 319–331. doi: 10.1016/j.molcel.2014.06.029

Sivanand, S., Viney, I., and Wellen, K. E. (2018). Spatiotemporal control of acetyl-coa metabolism in chromatin regulation. *Trends Biochem. Sci.* 43, 61–74. doi: 10.1016/j.tibs.2017.11.004

Stragand, J. J., Braunschweiger, P. G., Pollice, A. A., and Schiffer, L. M. (1979). Cell kinetic alterations in murine mammary tumors following fasting and refeeding. *Eur. J. Cancer* 15, 281–286. doi: 10.1016/0014-2964(79)90038-0

Su, X., Wellen, K. E., and Rabinowitz, J. D. (2016). Metabolic control of methylation and acetylation. *Curr. Opin. Chem. Biol.* 30, 52–60. doi: 10.1016/j.cbpa.2015.10.030

Symmons, O., Chang, M., Mellis, I. A., Kalish, J. M., Park, J., Suszták, K., et al. (2019). Allele-specific RNA imaging shows that allelic imbalances can arise in tissues through transcriptional bursting. *PLoS Genet.* 15:e1007874. doi: 10.1371/journal.pgen.1007874

Tajan, M., and Vousden, K. H. (2020). Dietary approaches to cancer therapy. *Cancer Cell* 37, 767–785. doi: 10.1016/j.ccell.2020.04.005

Tian, Y., Luo, C., Lu, Y., Tang, C., and Ouyang, Q. (2012). Cell cycle synchronization by nutrient modulation. *Integr. Biol. (Camb)* 4, 328–334. doi: 10.1039/c2ib00083k

Tokheim, C. J., Papadopoulos, N., Kinzler, K. W., Vogelstein, B., and Karchin, R. (2016). Evaluating the evaluation of cancer driver genes. *Proc. Natl. Acad. Sci. U.S.A.* 113, 14330–14335. doi: 10.1073/pnas.1616440113

Tomasetti, C., Li, L., and Vogelstein, B. (2017). Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. *Science* 355, 1330–1334. doi: 10.1126/science.aaf9011

Tomasetti, C., Marchionni, L., Nowak, M. A., Parmigiani, G., and Vogelstein, B. (2015). Only three driver gene mutations are required for the development of lung and colorectal cancers. *Proc. Natl. Acad. Sci. U.S.A.* 112, 118–123. doi: 10.1073/pnas.1421839112

Trefely, S., Lovell, C. D., Snyder, N. W., and Wellen, K. E. (2020). Compartmentalised acyl-CoA metabolism and roles in chromatin regulation. *Mol. Metab.* 38:100941. doi: 10.1016/j.molmet.2020.01.005

Tubiana, M. (1989). Tumor cell proliferation kinetics and tumor growth rate. *Acta Oncol.* 28, 113–121. doi: 10.3109/02841868909111193

Van de Peer, Y., Mizrachi, E., and Marchal, K. (2017). The evolutionary significance of polyploidy. *Nat. Rev. Genet.* 18, 411–424. doi: 10.1038/nrg.2017.26

van der Zee, J. (2002). Heating the patient: a promising approach? *Ann. Oncol.* 13, 1173–1184. doi: 10.1093/annonc/mdf280

Vigneau, S., Vinogradova, S., Savova, V., and Gimelbrant, A. (2018). High prevalence of clonal monoallelic expression. *Nat. Genet.* 50, 1198–1199. doi: 10.1038/s41588-018-0188-7

Wang, A., Huen, S. C., Luan, H. H., Yu, S., Zhang, C., Gallezot, J.-D., et al. (2016). Opposing effects of fasting metabolism on tissue tolerance in bacterial and viral inflammation. *Cell* 166, 1512–1525.e12. doi: 10.1016/j.cell.2016.07.026

Wang, P., González, M. C., Hidalgo, C. A., and Barabási, A.-L. (2009). Understanding the spreading patterns of mobile phone viruses. *Science* 324, 1071–1076. doi: 10.1126/science.1167053

Wei, M., Brandhorst, S., Shelehchi, M., Mirzaei, H., Cheng, C.-W., Budniak, J., et al. (2017). Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease. *Sci. Transl. Med.* 9:eaai8700. doi: 10.1126/scitranslmed.aai8700

Werner, B., Case, J., Williams, M. J., Chkhaidze, K., and Temko, D. (2019). Measuring single cell divisions in human cancers from multi-region sequencing data. *biorxiv.*[Preprint] doi: 10.1101/560243

Wray, G. A. (2007). The evolutionary significance of cis-regulatory mutations. *Nat. Rev. Genet.* 8, 206–216. doi: 10.1038/nrg2063

Wullschleger, S., Loewith, R., and Hall, M. N. (2006). TOR signaling in growth and metabolism. *Cell* 124, 471–484. doi: 10.1016/j.cell.2006.01.016

Wust, P., Hildebrandt, B., Sreenivasa, G., Rau, B., Gellermann, J., Riess, H., et al. (2002). Hyperthermia in combined treatment of cancer. *Lancet Oncol.* 3, 487–497. doi: 10.1016/s1470-2045(02)00818-5

Zalewski, P., Zawadka-Kunikowska, M., Słomko, J., Szrajda, J., Klawe, J. J., Tafil-Klawe, M., et al. (2014). Cardiovascular and thermal response to dry-sauna exposure in healthy subjects. *Physiol. J.* 2014:106049. doi: 10.1155/2014/106049

Zetterberg, A., and Larsson, O. (1985). Kinetic analysis of regulatory events in G1 leading to proliferation or quiescence of Swiss 3T3 cells. *Proc. Natl. Acad. Sci. U.S.A.* 82, 5365–5369. doi: 10.1073/pnas.82.16.5365

Zhang, J., Deng, Y., and Khoo, B. L. (2020). Fasting to enhance Cancer treatment in models: the next steps. *J. Biomed. Sci.* 27:58. doi: 10.1186/s12929-020-00651-0

Zhang, S., Zhang, H., Zhou, Y., Qiao, M., Zhao, S., Kozlova, A., et al. (2020). Allele-specific open chromatin in human iPSC neurons elucidates functional disease variants. *Science* 369, 561–565. doi: 10.1126/science.aay3983

**Conflict of Interest:** CG was a co-founder and has financial interests in Storyline Health Inc. www.storylinehealth.com, which is creating artificial intelligence technologies to improve behavioral health research and patient care, including for cancer patient care.

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 07 July 2021
doi: 10.3389/fevo.2021.660755

# Polyaneuploid Cancer Cell Dormancy: Lessons From Evolutionary Phyla

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Dormancy is a key survival strategy in many organisms across the tree of life. Organisms that utilize some type of dormancy (hibernation, aestivation, brumation, diapause, and quiescence) are able to survive in habitats that would otherwise be uninhabitable. Induction into dormant states is typically caused by environmental stress. While organisms are dormant, their physical activity is minimal, and their metabolic rates are severely depressed (hypometabolism). These metabolic reductions allow for the conservation and distribution of energy while conditions in the environment are poor. When conditions are more favorable, the organisms are then able to come out of dormancy and reengage in their environment. Polyaneuploid cancer cells (PACCs), proposed mediators of cancer metastasis and resistance, access evolutionary programs and employ dormancy as a survival mechanism in response to stress. Quiescence, the type of dormancy observed in PACCs, allows these cells the ability to survive stressful conditions (e.g., hypoxia in the microenvironment, transiting the bloodstream during metastasis, and exposure to chemotherapy) by downregulating and altering metabolic function, but then increasing metabolic activities again once stress has passed. We can gain insights regarding the mechanisms underlying PACC dormancy by looking to the evolution of dormancy in different organisms.

**Keywords: hibernation, dormancy, polyaneuploid cancer cells, cancer, evolution**

OPEN ACCESS

***Edited by:***
*Etienne Baratchart, Lund University, Sweden*

***Reviewed by:***
*Andriy Marusyk, Moffitt Cancer Center, United States*
*Anna K. Miller, Moffitt Cancer Center, United States*
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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *29 January 2021*
***Accepted:*** *15 June 2021*
***Published:*** *07 July 2021*

***Citation:***
*Kostecka LG, Pienta KJ and Amend SR (2021) Polyaneuploid Cancer Cell Dormancy: Lessons From Evolutionary Phyla. Front. Ecol. Evol. 9:660755. doi: 10.3389/fevo.2021.660755*

## INTRODUCTION: DORMANCY, AND CANCER

Metastatic cancer is resistant to almost all known systemic therapies and remains largely incurable (Pienta et al., 2020b). The inability to cure metastatic cancer leads to more than 10 million deaths globally per year (Bray et al., 2018). This resistance to therapy appears to be mediated, at least in part, by dormancy, a common survival strategy for many different organisms. Cancer cell dormancy occurs when cancer cells enter reversible cell cycle arrest known as quiescence (Yeh and Ramaswamy, 2015; Gao et al., 2017; Jahanban-Esfahlan et al., 2019; Recasens and Munoz, 2019). These dormant cancer cells lack proliferative and apoptotic markers but are still metabolically active and able to maintain essential cellular processes without continuous growth (Gao et al., 2017). Dormancy allows cancer cells the ability to survive new environments, initiate metastasis, become resistant to therapy, and evade immune detection (Recasens and Munoz, 2019). Cancer progression occurs when a dormant malignant cell gains the ability to re-enter cell cycle and restart uncontrolled proliferation. The ability to lay dormant during chemotherapy, stay metabolically

active, and eventually reinitiate proliferation enables cancer cell survival and results in observed therapy resistant recurrence (Pienta et al., 2020c, 2021).

New evidence suggests that the dormant cancer cell capable of surviving stress by entering a quiescent state is the polyaneuploid cancer cell (PACC) (Lopez-Sánchez et al., 2014; Pienta et al., 2021). PACCs [also referred to as polyploid giant cancer cells (PGCCs), multinucleated cancer cells, blastomere-like cancer cells, and osteoclast-like cancer cells] are enlarged cancer cells that have undergone whole-genome multiplication of their aneuploid genome (Erenpreisa et al., 2000, 2008; Mosieniak and Sikora, 2010; Zhang et al., 2014, 2015; Fei et al., 2015; Chen et al., 2019; Pienta et al., 2020c). Polyaneuploid cancer cells have been observed in cell lines, in patients, and in mouse models across virtually all cancer types (Virchow, 1860; Illidge et al., 2000; Erenpreisa et al., 2008; Puig, 2008; Zhang et al., 2013, 2014; Ogden et al., 2015; Mittal et al., 2017; Niu et al., 2017; Mirzayans et al., 2018; Amend et al., 2019; Chen et al., 2019). Polyaneuploid cancer cells are capable of forming in response to many different environmental/applied stressors such as hypoxia, lack of nutrients, changes in pH, chemotherapy, or radiation (Illidge et al., 2000; Makarovskiy et al., 2002; Erenpreisa et al., 2008; Puig, 2008; Lopez-Sánchez et al., 2014; Mittal et al., 2017; Mirzayans et al., 2018; Amend et al., 2019; Chen et al., 2019; Lin et al., 2019). PACCs can form by endoreplication, failed cytokinesis, and fusion (Illidge et al., 2000; Mirzayans et al., 2018; Amend et al., 2019; Chen et al., 2019; Lin et al., 2019). Cancer cells with the ability to become a PACC have the capability to alter metabolic functions to enter quiescence to avoid DNA damage, potentially providing a mechanism of therapy resistance. When stress is lifted, PACCs exit quiescence and can repopulate an aneuploid population by neosis (cell budding). A better understanding of cancer cell and PACC dormancy is critical in the quest to cure cancer. This review focuses on what we can learn from dormancy observed in ecology to better understand how polyaneuploid cancer cells alter their metabolism and survive in a dormant state while under stress.

## DORMANCY: A METABOLIC STRATEGY FOR SURVIVAL

Dormancy is a broadly used term to describe inactivity or lethargy (Navas and Carvalho, 2010). During dormancy, physical activity in organisms is minimal and metabolic rates can be altered or severely depressed (hypometabolism) (Navas and Carvalho, 2010; Mayer, 2016). Dormancy can be a response to various conditions such as circannual rhythm, temperature, or availability of resources (i.e., food and water) (Harlow, 1995; Lehmer et al., 2001; Navas and Carvalho, 2010). Dormancy can occur over short periods of time (less than a day), multiple days, an entire season, or, in extreme cases, even many years (Navas and Carvalho, 2010). While dormant organisms are physically inactive, they can still be aroused by disturbances without any major changes in their physiological state. There are many different forms of dormancy including hibernation (winter dormancy) (Geiser, 2013), aestivation (summer or dry season dormancy) (Storey and Storey, 2012), brumation (winter dormancy observed in ectotherms) (Wilkinson et al., 2017), diapause (period of suspended development in an insect, invertebrate, or mammal embryo in unfavorable conditions) (Denlinger, 2000; Tougeron, 2019), and quiescence (opportunistic inactivity observed in plants and cells) (**Table 1**) (Navas and Carvalho, 2010). During all of these dormant conditions organisms alter their metabolism to better survive unfavorable circumstances in their environment.

### Hibernation

One of the most well-known and commonly recognized forms of dormancy is hibernation (Geiser, 2013). Hibernation can be split into two categories: obligate hibernation and facultative hibernation (Chayama et al., 2016). Obligate hibernation, the more common form of hibernation, occurs when a mammal hibernates at the same time every year (e.g., arctic ground squirrels and dwarf lemurs) (Chayama et al., 2016). The other form of hibernation, facultative hibernation, occurs only when an organism faces stress in their environment (e.g., black-tailed prairie dogs) (Lehmer et al., 2001; Chayama et al., 2016). Hibernation is characterized by a reduction in body temperature, energy expenditure, and other physiological functions in mammals (Geiser, 2013). These changes in metabolic functions allow for the conserved resources to be utilized throughout a multiday torpor (inactivity). Multiday torpor was originally hypothesized to only occur in winter due to the cold weather, however, it has since been revealed that it is most likely due to the lack of resources available during winter rather than the weather itself (Geiser, 2013). Hibernating species have evolved the ability to enter torpor to conserve energy by reducing their metabolic states in order to survive. Without evolving the ability to hibernate, many organisms would not have survived the environments they live in.

#### Obligate Hibernation

The majority of ground squirrels are obligate hibernators, including the arctic ground squirrel. Obligate hibernation allows arctic ground squirrels to survive harsh winters in Alaska despite sub-zero temperatures, frozen soil, little to no food available, and near complete darkness (Loren and Barnes, 1999). Obligate hibernators, like the arctic ground squirrel, have endogenously timed annual dormancy in winter where they generate energy reserves during the warmer months and then that energy is conserved through a large reduction of basal metabolic rate, heart rate, blood flow, and temperature while they hibernate (Loren and Barnes, 1999; Singhal et al., 2020). Arctic ground squirrels can hibernate for up to 8 months out of every year and during this hibernation they lower their body temperature to adopt the lowest body temperature ever measured in a mammal (-2.9°C) (Loren and Barnes, 1999; Buck et al., 2008; Richter et al., 2015; Singhal et al., 2020; Rice et al., 2020). Arctic ground squirrels recycle broken down nutrients while in hibernation to enable survival (Rice et al., 2020). Muscle is broken down and the free nitrogen generated can be converted into necessary amino acids (Rice et al., 2020). Using those amino acids, the squirrels can synthesize new proteins necessary for continued survival

**TABLE 1 |** Metabolic characteristics of dormancy in nature and in PACCs.

| Type of dormancy | Ecologic example | Organism metabolic characteristics | PACC metabolic similarities |
|---|---|---|---|
| Obligate hibernation | Arctic ground squirrel | • Occurs annually (winter)<br>• Metabolic depression<br>• Recycling of broken-down materials for energy | • High autophagy levels: recycling of broken-down intracellular contents for energy |
| Facultative hibernation | Black-tailed prairie dog | • Only occurs under stress<br>• Metabolic depression<br>• Low lipid peroxidation rates | • Only occurs under stress<br>• High lipid levels |
| Aestivation | *Helix aspersa* | • Metabolic depression<br>• Reduction in macromolecule synthesis and degradation | • Altered metabolism |
| Brumation | Tiger salamander | • Can occur annually or under stress (depends on the species)<br>• Metabolic depression | • Altered metabolism |
| Diapause | *Bombyx mori* | • Only occurs under stress<br>• Metabolic depression<br>• Low behavioral activity<br>• Slowing of growth<br>• Reproductive functional arrest | • Slowing of growth<br>• Low motility<br>• Halt in cell division |
| Quiescence | *S. cerevisiae* | • Occurs under stress<br>• Widened cell wall<br>• Sequestering protein<br>• Transcriptional shut down<br>• Increase in size<br>• Halt in cell division | • Enlarged cell structure<br>• High lipid levels<br>• Halt in cell division |

during their torpor without needing to ingest new nutrients from the environment (Rice et al., 2020). Additionally, arctic ground squirrels often wake from their hibernation while conditions in Alaska are still harsh and still have enough energy reserve leftover to sustain life until conditions improve and they are able to successfully forage for food (Loren and Barnes, 1999). This means that they strictly only dispense energy while in hibernation when completely necessary so as not to waste energy. This annual hibernation to avoid the harsh winters allows arctic ground squirrels to survive and populate a habitat which they would otherwise be unable to survive in.

### Facultative Hibernation

Black-tailed prairie dogs, facultative hibernators, are unlike obligate hibernators as they only enter a shallow torpor in times of stress rather than entering an annual multi-day torpor. This shallow torpor is most commonly induced when black-tailed prairie dogs are severely cold or deprived of food and water (Harlow, 1995; Lehmer et al., 2001; Harlow and Frank, 2001). Similar to obligate hibernators, black-tailed prairie dogs experience a drop in body temperature and metabolic rate during their short term torpor (Geiser, 2013). The physiological advantages for adapting to be a facultative hibernator are still not well known, but it is hypothesized that natural selection likely favored facultative hibernation as black-tailed prairie dogs have lower lipid peroxidation rates, allowing them to conserve fat for extended periods of time (Harlow and Frank, 2001). Black-tailed prairie dogs share a common ancestor with white-tailed prairie dogs yet white-tailed prairie dogs remained obligate hibernators while black-tailed prairie dogs evolved to be facultative hibernators (Harlow, 1995). Some hypotheses of why black-tailed prairie dogs evolved to be facultative hibernators include (1) a lengthened growing season and a higher abundance of food in the great plains reducing the need for long term hibernation, (2) a lack of refuge from their most common predator, the black-footed-ferret, forced them to evolve to hibernate less, and (3) the black-tailed prairie dog conserves fat rather than protein during fasting which could reduce its ability to successfully hibernate for long periods of time (Harlow, 1995). While the true reason for this evolution is still unknown, it is interesting to note that evolving to be facultative hibernators has allowed black-tailed prairie dogs to only hibernate when absolutely necessary for survival, not on an annual basis.

## Aestivation

Aestivation, while similar to hibernation, occurs during the warmer months of the year instead of during the winter (Navas and Carvalho, 2010). Aestivation is also known as "light" hibernation as these organisms are able to wake from their dormant state very quickly after the stress is removed. Ectotherms, organisms that are dependent on external sources of body heat (i.e., "cold-blooded"), undergo an intrinsic metabolic depression in which their metabolic rate declines to about 10–20% of their resting metabolic rate at the same body temperature, while endotherms, organisms capable of the internal generation of heat (i.e., "warm-blooded") undergo a fundamental physiological change in body temperature, reduction in metabolic rate, and water loss (Navas and Carvalho, 2010). *Helix aspersa*, commonly known as the garden snail, is an example of an ectotherm that performs aestivation (Pedler et al., 1996). When overheated or underfed these snails are able to create a seal on their shell to prevent any residual moisture from leaving their body (Pedler et al., 1996). The snails then enter aestivation for long or short periods of time to survive whatever harsh conditions they have encountered (Pedler et al., 1996). During this period the snail only allocates energy to the

most necessary processes for survival. For example, energy is not allocated to reproductive efforts. While hibernation during winter is the most common form of torpor known, aestivation provides animals with a survival strategy during the summer months. The utilization of dormancy and an altered metabolism to survive harsh heat, drought, and lack of food allows these animals to live and populate in an environment that would otherwise be deadly to them.

## Brumation

Brumation is winter dormancy performed by ectotherms (Wilkinson et al., 2017; Kundey et al., 2018). While (obligate or facultative) hibernating mammals may experience regular small arousals from torpidity during the winter months, ectotherms undergoing brumation are dependent upon the temperature of their environment and stay dormant until temperatures rise again (Wilkinson et al., 2017; Kundey et al., 2018). An example of an ectotherm that undergoes brumation is the tiger salamander (Kundey et al., 2018). If temperature is constant and within a normal range for the salamanders they will not undergo brumation at any point during the year. However, if the salamanders live in an area where the temperature drastically drops at any point in the year they automatically enter brumation to survive the temperature change (Kundey et al., 2018). Tiger salamanders are also able to enter into aestivation during summer on days that are extremely hot, but their aestivation periods are typically much shorter than their brumation periods (Kundey et al., 2018). Additionally, multiple studies have shown that entering and exiting brumation does not result in any change in memory retention, meaning that as soon as tiger salamanders come out of brumation they remember exactly where to find food and how to find a mate (Wilkinson et al., 2017; Kundey et al., 2018). Tiger salamanders' unique adaptions to enter brumation allow them to survive harsh winters or summers that would otherwise eliminate them from the environment.

## Diapause

Diapause is a form of developmental arrest in insects that is equivalent to hibernation in mammals (Denlinger, 2000). Diapause is characterized by low metabolic activity, low behavioral activity, morphogenesis and reproductive functional arrest, and the slowing of growth (Denlinger, 2000; Tougeron, 2019). Diapause is obligatory in some species of insects and facultative in others (Denlinger, 2000). Diapause is induced by abiotic cues that indicate the onset of adverse conditions in the environment (Denlinger, 2000; Antonova-Koch et al., 2013). While diapause most commonly occurs during the winter, it can also occur during summer in months of extreme heat when there is a lack of resources (Denlinger, 2000). The arrest induced by diapause occurs in species-specific life stages (Antonova-Koch et al., 2013; Tougeron, 2019). For example, in the silk moth *Bombyx mori,* diapause occurs early in embryonic development, while in the gypsy moth *Lymantria dispar,* diapause occurs at the completion of embryonic development (Denlinger, 2000). Diapause allows insects at different life stages to halt growth and activity in adverse environments to enhance their survival. Without evolving to perform diapause, insects may have only been able to populate very specific regions of the world making them susceptible to predation and overcrowding.

## Quiescence

Cellular quiescence, also known as the G0 stage in cell cycle, is defined as reversible proliferation arrest. Unicellular organisms as well as individual cells of various prokaryotic and eukaryotic microorganisms can survive in a quiescent state for long periods of time (days, months, or years) without added nutrients (Gray et al., 2004). Unicellular organisms and cells in complex organisms utilize and spend a part of their life in quiescence (Sagot and Laporte, 2019). Entry into quiescence is associated with dramatic decreases and changes in metabolic activities (Sagot and Laporte, 2019). While metabolism may slow and change while these cells are in a non-proliferative state, these cells are not completely inactive as they still perform basic maintenance for survival. Unicellular organisms are more frequently quiescent and can remain in a quiescent state waiting for signals such as temperature, oxygen, or nutrients to exit quiescence and begin proliferating again (Sagot and Laporte, 2019). An example of an organism that regularly utilizes quiescence is *Saccharomyces cerevisiae,* a species of yeast (Wloch-Salamon et al., 2017; Sagot and Laporte, 2019). When stressed or nutrient deprived it has been shown that around 75% of a stationary-phase culture of *S. cerevisiae* will enter into a quiescent state (Wloch-Salamon et al., 2017). *S. cerevisiae* quiescent cells differ from non-quiescent cells in the same culture as they develop a widened cell wall (Aragon et al., 2008), increase storage of carbohydrates (Aragon et al., 2008), sequester proteins (Suresh et al., 2015), cluster telomeres (Tjaden, 2015), and undergo transcriptional shut down (McKnight et al., 2015). Additionally, it has been shown that cell volume influences quiescence exit efficiency (Sagot and Laporte, 2019). *S. cerevisiae* cells exiting quiescence must reach a critical size before they are able to enter the S phase of cell cycle and thus larger quiescent yeast generally emit buds faster than smaller quiescent yeast (Laporte et al., 2018). Quiescence is a unique form of cellular dormancy that allows unicellular and multicellular organisms the capability to survive stress in many various forms by essentially shutting down reproductive efforts and lowering metabolic functions until the stress is removed. *Saccharomyces cerevisiae* provide a unique model that can be used to better understand cancer cell metabolism during dormancy.

# DISCUSSION: UNDERSTANDING DIFFERENT TYPES OF DORMANCY PROVIDES INSIGHT INTO DORMANT PACC METABOLISM

All types of hibernation give organisms a mechanism of survival in unfavorable environments. Common themes for all types of dormancy are lowered levels of activity, little to no reproductive efforts, lowered intake of nutrients, and reduced metabolic rates. PACCs require a survival mechanism and altering their metabolism while in a dormant state is a possible mechanism

to survive stresses such as chemotherapy. Understanding and comparing the different types of dormancy in nature provides insight into how altered PACC metabolism during dormancy is helping these cells survive and provides a possible opportunity for therapeutic intervention.

Throughout evolution, organisms have adapted many different metabolic strategies for survival. A hallmark of cancer is reprogramming energy metabolism (Hanahan and Weinberg, 2011). One of the most well characterized metabolic changes in cancer cells is the Warburg effect: that cancer cells preferentially utilize glycolysis to generate the majority of their ATP, even while in aerobic conditions (Warburg, 1930; Hanahan and Weinberg, 2011). When cancer cells are stressed their metabolic signatures can be further altered. Cancer cells undergoing stress such as starvation, radiation, or chemotherapy are known to have an altered metabolism compared to their non-stress counterparts, with evidence of accumulation of lipids and an increase in autophagy to survive (Hanahan and Weinberg, 2011).

To study the metabolism of dormant cancer cells under stress, the dormant population must first be identified. Upon treatment of a cancer cell population with chemotherapy, an emergence of physically enlarged cells with high genomic content are observed *in vitro* (Erenpreisa et al., 2008; Zhang et al., 2014; Amend et al., 2019; Pienta et al., 2020a,c). These cells, defined as PACCs, exhibit temporary polyploidization of their aneuploid genome while simultaneously altering their metabolism to survive environmental stress (Pienta et al., 2020a,c). Following polyploidization, PACCs halt cell proliferation, entering a dormant state (Pienta et al., 2021). Eventually, when stress is removed, PACCs exit the dormant state and generate progeny to establish a recurrence (Puig, 2008; Zhang et al., 2013; Amend et al., 2019). PACCs are a transient state of cancer cells that are hypothesized to utilize dormancy and an altered metabolism for survival (Pienta et al., 2020a,c). While under stress, PACCs appear to utilize different metabolic functions to survive, but the exact PACC metabolic signature has yet to be defined (Sirois et al., 2019). Cellular quiescence is associated with low metabolic rates, altered glucose metabolism, lipid accumulation, and an activation of autophagy to provide nutrients for survival (Valcourt et al., 2012). The quiescent state in PACCs can be compared to dormancy in organisms that are able to remain in an inactive state for long periods of time under stress for enhanced survival. Comparing dormant PACC metabolism to the metabolism of other dormant organisms across the tree of life may provide valuable insight into PACC biology and survival mechanisms.

Different types of dormancy have evolved for survival of organisms across multiple kingdoms of life. The key component in all different types of dormancy is altered (though not necessarily diminished) metabolic activity for prolonged survival without further intake of necessary nutrients. Similarities can be observed between all types of dormancy and PACC dormancy (**Table 1**). For example, while in obligate hibernation, the arctic ground squirrel recycles cellular components for energy so that intake of nutrients during hibernation is unnecessary. Facultative hibernation highlights that dormancy is not exclusively an annual or periodic event but can occur near-immediately as needed for survival. Quiescence allows yeast the ability to exit the active cell cycle during stress to conserve resources until the stress is removed and they can begin proliferating again. All of these strategies are key survival mechanisms that cancer cells seem to access.

Obligate hibernators hibernate annually every winter. During obligate hibernation arctic ground squirrels can recycle and reuse select cellular components to make up for their lack of nutrient intake. Arctic ground squirrel skeletal muscle is broken down and the free nitrogen generated is converted into necessary amino acids which are synthesized into proteins that are essential for continued survival during hibernation (Rice et al., 2020). This metabolic survival mechanism can be compared to elevated autophagy in cancer cells (**Table 1**). When cancer cells are stressed, autophagy is induced. Autophagy is the process in which cells engulf and break down portions of their cytoplasm to be recycled for future use. This process can generate a high level of fatty acids and energy for the cells. PACCs that enter quiescence due to chemotherapy treatment are under high levels of stress and are hypothesized to have increased autophagy levels (Dudkowska et al., 2021). High levels of autophagy could give PACCs a metabolic advantage during quiescence by slowly breaking down cellular components for energy instead of importing nutrients from the toxic environment. This metabolic "recycling" allows arctic ground squirrels and PACCs the ability to better survive dormancy without exerting excess energy to obtain more resources and limiting risk from the harsh environment.

Facultative hibernation allows mammals the advantage of entering torpor only when it is necessary. This allows mammals to continue foraging, hunting, and mating until the moment they become too stressed by changes in their environment and they must decrease metabolic activities and enter torpor to survive. Black-tailed prairie dogs have been shown to have lower lipid peroxidation rates while undergoing torpor (Harlow, 1995). This implies a storage of lipids and fats, but not a high usage of them, indicating the hibernating black-tailed prairie dogs are either using other sources of energy to survive, slowly breaking

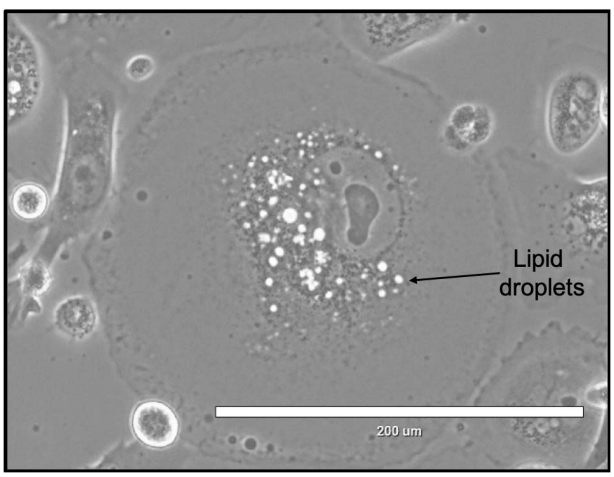


**FIGURE 1 |** PACCs accumulate lipid droplets. This image shows a single PACC with a high level of lipids (black arrow) surrounding the nucleus.

down these lipids for energy to ensure they have enough energy for long term survival, or they are sequestering toxins in these lipids to protect other cells in the body from damage. Cancer cells mimic facultative hibernation when conditions are bad (e.g., chemotherapy is introduced to the environment) by forming PACCs which are in a metabolically depressed quiescent state (**Table 1**). While in a quiescent state, PACCs appear to have high number of lipid droplets that are not quickly degraded (**Figure 1**) (Sirois et al., 2019). These lipids could be sequestering toxins or they could be saved as energy reserves for when PACCs exit quiescence and proliferate again. PACCs and black-tailed prairie dogs only enter dormancy when absolutely necessary to survive stressors that would otherwise destroy the rest of the population. The ability to only lay dormant when absolutely necessary allows PACCs and black-tailed prairie dogs to live and inhabit environments that may have been deadly to them otherwise.

Quiescence is a key survival strategy for many unicellular organisms. Quiescence allows yeast to survive nutrient deprived and other harsh environments. Quiescent cells are found all throughout the human body and are vital for normal tissue homeostasis (Coller, 2011; Yao, 2014). Infrequent cell cycle (entering and exiting quiescence) has been described as a fundamental mechanism that can contribute to the evolution of therapy resistance in cancer (Coward and Harding, 2014; Donovan et al., 2014). Cellular quiescence can provide metabolic adaptions as well as protection against stress and toxicities which is important for long-lived cells and, unfortunately, for cancer cells (Yao, 2014). It is hypothesized that PACCs enter into quiescence and alter their metabolism as a survival strategy when they encounter stress (e.g., hypoxia, chemotherapy or radiation) and then re-enter the cell cycle when the stress is lifted (Erenpreisa et al., 2008; Lopez-Sánchez et al., 2014;

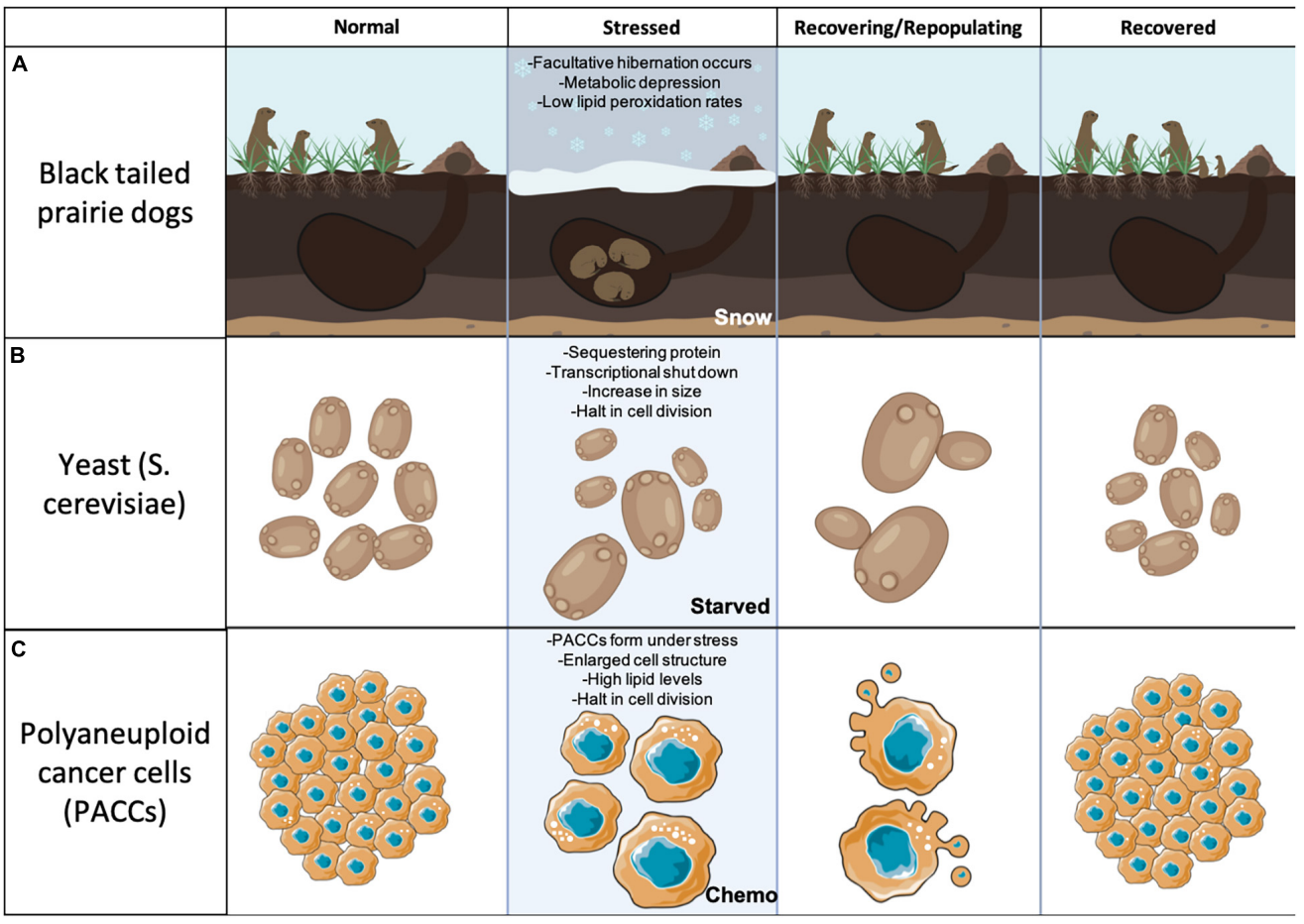


**FIGURE 2 |** Parallel relationships between black-tailed prairie dogs, *S. cerevisiae*, and PACCs. **(A)** Black-tailed prairie dogs in a normal, non-stressed environment are active in the environment. When stress (snow) is applied the black-tailed prairie dogs enter facultative hibernation. When the snow goes away the black-tailed prairie dogs are able to exit their dormant state, become active again, and reproduce. **(B)** *S. cerevisiae* are first shown in a normal environment. When stressed (starved) they enter into a quiescent state and some become enlarged. When stress is released they are able to exit quiescence and repopulate utilizing budding. **(C)** Cancer cells in a normal environment are constantly proliferating. When stress (chemotherapy) is applied many of these cells undergo failed cytokinesis or endoreplication and become PACCs. PACCs are larger than the original cancer cells and enter into a quiescent non-proliferative state, and contain high levels of lipid droplets. When stress is released PACCs are able to undergo various forms of division (i.e., restart reproductive efforts) such as neosis or asymmetrical division to produce new, smaller progeny that are the same size as the original cell population.

Pienta et al., 2020b). Some parallels of yeast and PACC quiescence seem to be that larger quiescent yeast cells are more successful at survival and exiting quiescence while PACCs utilize larger size to house additional cellular machinery to metabolize toxins as well as access additional nutrients though autophagy (**Table 1**). Additionally, quiescent yeast sequester proteins and undergo transcriptional shut down to survive. Similarly, PACCs appear to have high levels of lipids (Sirois et al., 2019) which means they are most likely harboring fat to use during their dormant state as well as not proliferating to save energy.

Analyzing parallel relationships between dormant organisms can give us insight into how PACCs survive dormancy. **Figure 2** demonstrates the parallel relationships between two different types of organisms that utilize dormancy (black-tailed prairie dogs and yeast) and PACCs. Black-tailed prairie dogs feed on grass around their burrows to put on weight for winter months when food is scarce and potential hibernation may occur. Only when a stressor is introduced (snow/no food) will the black-tailed prairie dogs actually begin facultative hibernation for survival. When the environment is favorable again the black-tailed prairie dogs are able to exit their temporary torpor and resume normal daily activities and reproductive efforts (**Figure 2A**). Similarly, when *S. cerevisiae* are starved, the majority of the population becomes quiescent and some individuals become enlarged. As stated earlier, recovering, larger *S. cerevisiae* are more successful at exiting quiescence. The budded progeny of *S. cerevisiae* return to the same size as the original *S. cerevisiae* population (**Figure 2B**). Lastly, when in a stable environment, cancer cells are continuously proliferating. However, when stressed with chemotherapy, typical cancer cells become PACCs through failed cytokinesis, fusion, or endoreplication (Erenpreisa et al., 2008; Amend et al., 2019; Lin et al., 2019; Chen et al., 2019). Polyaneuploid cancer cell are identified as large cells with a high of genomic content. Under stress PACCs are the majority of the population and enter into a quiescent state to survive. In this quiescent state PACCs contain high levels of lipids (Sirois et al., 2019), they do not reproduce (Pienta et al., 2021), and they are physically enlarged, similar to the enlarged *S. cerevisiae*. When stress is released, PACCs are able to undergo division (i.e., restart reproductive efforts) in the form of neosis (cell budding) to produce new, smaller progeny that are the same size as the original cell population (**Figure 2C**) (Sundaram et al., 2004; Zhang et al., 2013; Amend et al., 2019).

All three of these examples demonstrate dormancy aiding in the survival of these groups. During dormancy all of these groups alter their metabolism to better survive in a dormant state. Whether it be black-tailed prairie dogs that only enter torpor when absolutely necessary for survival, or *S. cerevisiae* growing in size to aid in their eventual repopulation, there are parallels to PACC metabolism.

## CONCLUSION

Despite increasing knowledge regarding molecular mechanisms that drive cancer cell dormancy and their potential targets, the best therapeutic approach to targeting dormancy still remains unclear. Evolution provides examples of how various organisms' metabolic activities are altered during dormancy to enhance survival. Learning from models in nature like arctic ground squirrels (breaking down and reusing nutrients), black-tailed prairie dogs (low lipid peroxidation rates), and yeast (larger size assists with exiting quiescence) can help us elucidate PACC metabolism during dormancy. Learning more about PACC metabolism in dormancy may be the key to learning how to target these cells while in a dormant state. For example, the next steps point to targeting lipid metabolism or autophagy initiation which may be key factors to PACC survival in a quiescent state. Disrupting PACC metabolism in a quiescent state will ultimately lead to apoptosis of these cells. Successfully targeting these cells will provide a critical therapeutic opportunity to reduce metastatic chemotherapy-resistant tumor burden.

## AUTHOR CONTRIBUTIONS

LK: writing – original draft preparation and conceptualization. KP: funding acquisition and writing – reviewing and editing. SA: conceptualization, funding acquisition, and writing – reviewing and editing. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the US Department of Defense CDMRP/PCRP (W81XWH-20-10353), the Patrick C. Walsh Prostate Cancer Research Fund and the Prostate Cancer Foundation to SA; and NCI grants U54CA143803, CA163124, CA093900, and CA143055, and the Prostate Cancer Foundation to KP. This work was also supported by the William and Carolyn Stutt Research Fund, Ronald Rose, MC Dean, Inc., William and Marjorie Springer, Mary and Dave Stevens, Louis Dorfman, and the Jones Family Foundation.

## REFERENCES

Amend, S. R., Torga, G., Lin, K. C., Kostecka, L. G., de Marzo, A., Austin, R. H., et al. (2019). Polyploid giant cancer cells: unrecognized actuators of tumorigenesis, metastasis, and resistance. *Prostate* 79, 1489–1497. doi: 10.1002/pros.23877

Antonova-Koch, Y., Riehle, M. A., Arik, A. J., and Brown, M. R. (2013). "Chapter 38 - Insulin-Like Peptides," in *Handbook of Biologically Active Peptides 2nd Edition*, ed. A. Kastin (Amsterdam: Elsevier),

Aragon, A. D., Rodriguez, A. L., Meirelles, O., Roy, S., Davidson, G. S., Tapia, P. H., et al. (2008). Characterization of Differentiated Quiescent and Nonquiescent Cells in Yeast Stationary-Phase Cultures. *Mol. Biol. Cell*19, 1271–1280. doi: 10.1091/mbc.e07-07-0666

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 68, 394–424. doi: 10.3322/caac.21492

Buck, C. L., Breton, A., Kohl, F., and Tøien, O. (2008). "Overwinter body temperature patterns in free-living arctic squirrels (*Spermophilus parryii*)," in *Hypometabolism in animals: torpor hibernation and cryobiology*, eds B. G. Lovegrove and A. E. McKechnie (Pietermaritzburg: University of KwaZulu-Natal), 317–326.

Chayama, Y., Ando, L., Tamura, Y., Miura, M., and Yamaguchi, Y. (2016). Decreases in body temperature and body mass constitute pre-hibernation remodelling in the Syrian golden hamster, a facultative mammalian hibernator. *R. Soc. Open Sci.* 3:160002. doi: 10.1098/rsos.160002

Chen, J., Niu, N., Zhang, J., Qi, L., Shen, W., Donkena, K. V., et al. (2019). Polyploid Giant Cancer Cells (PGCCs): the Evil Roots of Cancer. *Curr. Cancer Drug Targets* 19, 360–367. doi: 10.2174/1568009618666180703154233

Coller, H. A. (2011). The Essence of Quiescence. *Science* 334, 1074–1075. doi: 10.1126/science.1216242

Coward, J., and Harding, A. (2014). Size Does Matter: why Polyploid Tumor Cells are Critical Drug Targets in the War on Cancer. *Front. Oncol.* 4:123. doi: 10.3389/fonc.2014.00123

Denlinger, D. L. (2000). "Chapter 18 - Molecular Regulation of Insect Diapause," in *Cell and Molecular Response to Stress*, eds K. B. Storey and J. M. Storey (Amsterdam: Elsevier), 259–275. doi: 10.1016/S1568-1254(00)80020-0

Donovan, P., Cato, K., Legaie, R., Jayalath, R., Olsson, G., Hall, B., et al. (2014). Hyperdiploid tumor cells increase phenotypic heterogeneity within Glioblastoma tumors. *Mol. BioSyst.* 10, 741–758. doi: 10.1039/C3MB70484J

Dudkowska, M., Staniak, K., Bojko, A., and Sikora, E. (2021). "Chapter Five - The role of autophagy in escaping therapy-induced polyploidy/senescence," in *Advances in Cancer Research*, eds D. A. Gewirtz and P. B. Fisher (Cambridge: Academic Press), 209–247. doi: 10.1016/bs.acr.2021.01.004

Erenpreisa, J., Ivanov, A., Wheatley, S. P., Kosmacek, E. A., Ianzini, F., Anisimov, A. P., et al. (2008). Endopolyploidy in irradiated p53-deficient tumour cell lines: persistence of cell division activity in giant cells expressing Aurora B- kinase. *Cell Biol. Int.* 32, 1044–1056. doi: 10.1016/j.cellbi.2008.06.003

Erenpreisa, J. A., Cragg, M. S., Fringes, B., Sharakhov, I., and Illidge, T. M. (2000). Release of Mitotic Descendants by Giant Cells from Irradiated Burkitt's Lymphoma Cell Lines. *Cell Biol. Int.* 24, 635–648. doi: 10.1006/cbir.2000.0558

Fei, F., Zhang, D., Yang, Z., Wang, S., Wang, X., Wu, Z., et al. (2015). The number of polyploid giant cancer cells and epithelial-mesenchymal transition-related proteins are associated with invasion and metastasis in human breast cancer. *J. Exp. Clin. Cancer Res.* 34:158. doi: 10.1186/s13046-015-0277-8

Gao, X., Zhang, M., Tang, Y., and Liang, X. (2017). Cancer cell dormancy: mechanisms and implications of cancer recurrence and metastasis. *Onco Targets Ther.* 10, 5219–5228. doi: 10.2147/OTT.S140854

Geiser, F. (2013). Hibernation. *Curr. Biol.* 23, R188–R193. doi: 10.1016/j.cub.2013.01.062

Gray, J. V., Petsko, G. A., Johnston, G. C., Ringe, D., Singer, R. A., and Werner-Washburne, M. (2004). Sleeping Beauty': quiescence in Saccharomyces cerevisiae. *Microbiol. Mol. Biol. Rev.* 68:20.

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of Cancer: the Next Generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Harlow, H. J. (1995). Fasting Biochemistry of Representative Spontaneous and Facultative Hibernators: the White-Tailed Prairie Dog and the Black-Tailed Prairie Dog. *Physiol. Zool.* 68, 915–934. doi: 10.1086/physzool.68.5.30163938

Harlow, H. J., and Frank, C. L. (2001). The role of dietary fatty acids in the evolution of spontaneous and facultative hibernation patterns in prairie dogs. *J. Comp. Physiol. B* 171, 77–84. doi: 10.1007/s003600000148

Illidge, T. M., Cragg, M. S., Fringes, B., Olive, P., and Erenpreisa, J. A. (2000). Polyploid giant cells provide a survival mechanism for p53 mutant cells after DNA damage. *Cell Biol. Int.* 24, 621–633. doi: 10.1006/cbir.2000.0557

Jahanban-Esfahlan, R., Seidi, K., Manjili, M. H., Jahanban-Esfahlan, A., Javaheri, T., and Zare, P. (2019). Tumor Cell Dormancy: threat or Opportunity in the Fight against Cancer. *Cancers* 11:1207. doi: 10.3390/cancers11081207

Kundey, S. M. A., Lessard, A., Fitz, A., and Panwar, M. (2018). Tiger salamanders' (Ambystoma tigrinum) response retention and usage of visual cues following brumation. *Behav. Processes* 157, 502–508. doi: 10.1016/j.beproc.2018.06.008

Laporte, D., Jimenez, L., Gouleme, L., and Sagot, I. (2018). Yeast quiescence exit swiftness is influenced by cell volume and chronological age. *Microb. Cell* 5, 104–111. doi: 10.15698/mic2018.02.615

Lehmer, E. M., Van Horne, B., Kulbartz, B., and Florant, G. L. (2001). Facultative Torpor in Free-Ranging Black-Tailed Prairie Dogs (*Cynomys Ludovicianus*). *J. Mammal.* 82, 551–557. doi: 10.1644/1545-15422001082<0551:FTIFRB<2.0.CO;2

Lin, K.-C., Torga, G., Sun, Y., Axelrod, R., Pienta, K. J., Sturm, J. C., et al. (2019). The role of heterogeneous environment and docetaxel gradient in the emergence of polyploid, mesenchymal and resistant prostate cancer cells. *Clin. Exp. Metastasis* 36, 97–108. doi: 10.1007/s10585-019-09958-1

Lopez-Sánchez, L. M., Jimenez, C., Valverde, A., Hernandez, V., Peñarando, J., Martinez, A., et al. (2014). CoCl2, a Mimic of Hypoxia, Induces Formation of Polyploid Giant Cells with Stem Characteristics in Colon Cancer. *PLoS One* 9:e99143. doi: 10.1371/journal.pone.0099143

Loren, C., and Barnes, B. M. (1999). Annual cycle of body composition and hibernation in free-living arctic ground squirrels. *J. Mammal.* 80:13.

Makarovskiy, A. N., Siryaporn, E., Hixson, D. C., and Akerley, W. (2002). Survival of docetaxel-resistant prostate cancer cells in vitro depends on phenotype alterations and continuity of drug exposure. *Cell. Mol. Life Sci.* 59, 1198–1211. doi: 10.1007/s00018-002-8498-3

Mayer, W. V. (2016). "Dormancy," in *Encyclopedia Britannica* Available online at: https://www.britannica.com/science/dormancy (accessed December 1, 2020).

McKnight, J. N., Boerma, J. W., Breeden, L. L., and Tsukiyama, T. (2015). Global Promoter Targeting of a Conserved Lysine Deacetylase for Transcriptional Shutoff During Quiescence Entry. *Mol. Cell* 59, 732–743. doi: 10.1016/j.molcel.2015.07.014

Mirzayans, R., Andrais, B., and Murray, D. (2018). Roles of Polyploid/Multinucleated Giant Cancer Cells in Metastasis and Disease Relapse Following Anticancer Treatment. *Cancers* 10:118. doi: 10.3390/cancers10040118

Mittal, K., Donthamsetty, S., Kaur, R., Yang, C., Gupta, M. V., Reid, M. D., et al. (2017). Multinucleated polyploidy drives resistance to Docetaxel chemotherapy in prostate cancer. *Br. J. Cancer* 116, 1186–1194. doi: 10.1038/bjc.2017.78

Mosieniak, G., and Sikora, E. (2010). Polyploidy: the link between senescence and cancer. *Curr. Pharm. Des.* 16, 734–740. doi: 10.2174/138161210790883714

Navas, C. A., and Carvalho, J. E. (eds) (2010). *Aestivation*. Berlin: Springer.

Niu, N., Mercado-Uribe, I., and Liu, J. (2017). Dedifferentiation into blastomere-like cancer stem cells via formation of polyploid giant cancer cells. *Oncogene* 36, 4887–4900. doi: 10.1038/onc.2017.72

Ogden, A., Rida, P. C. G., Knudsen, B. S., Kucuk, O., and Aneja, R. (2015). Docetaxel-induced polyploidization may underlie chemoresistance and disease relapse. *Cancer Lett.* 367, 89–92. doi: 10.1016/j.canlet.2015.06.025

Pedler, S., Fuery, C. J., Withers, P. C., Flanigan, J., and Guppy, M. (1996). Effectors of metabolic depression in an estivating pulmonate snail (Helix aspersa): whole animal and in vitro tissue studies. *J. Comp. Physiol. B* 166, 375–381. doi: 10.1007/BF02336920

Pienta, K. J., Hammarlund, E. U., Austin, R. H., Axelrod, R., Brown, J. S., and Amend, S. R. (2020a). Cancer cells employ an evolutionarily conserved polyploidization program to resist therapy. *Semin. Cancer Biol.* doi: 10.1016/j.semcancer.2020.11.016 [Online ahead of print]

Pienta, K. J., Hammarlund, E. U., Axelrod, R., Amend, S. R., and Brown, J. S. (2020b). Convergent Evolution, Evolving Evolvability, and the Origins of Lethal Cancer. *Mol. Cancer Res.* 18, 801–810. doi: 10.1158/1541-7786.MCR-19-1158

Pienta, K. J., Hammarlund, E. U., Axelrod, R., Brown, J. S., and Amend, S. R. (2020c). Poly-aneuploid cancer cells promote evolvability, generating lethal cancer. *Evol. Appl.* 13, 1626–1634. doi: 10.1111/eva.12929

Pienta, K. J., Hammarlund, E. U., Brown, J. S., Amend, S. R., and Axelrod, R. M. (2021). Cancer recurrence and lethality are enabled by enhanced survival and reversible cell cycle arrest of polyaneuploid cells. *Proc. Natl. Acad. Sci. U. S. A.* 118:e2020838118. doi: 10.1073/pnas.2020838118

Puig, P.-E. (2008). Tumor cells can escape DNA-damaging cisplatin through DNA endoreduplication and reversible polyploidy. *Cell Biol. Int.* 32, 1031–1043.

Recasens, A., and Munoz, L. (2019). Targeting Cancer Cell Dormancy. *Trends Pharmacol. Sci.* 40, 128–141. doi: 10.1016/j.tips.2018.12.004

Rice, S. A., Ten Have, G. A. M., Reisz, J. A., Gehrke, S., Stefanoni, D., Frare, C., et al. (2020). Nitrogen recycling buffers against ammonia toxicity from skeletal muscle breakdown in hibernating arctic ground squirrels. *Nat. Metab.* 2:12. doi: 10.1038/s42255-020-00312-4

Richter, M. M., Williams, C. T., Lee, T. N., Tøien, Ø, Florant, G. L., Barnes, B. M., et al. (2015). Thermogenic capacity at subzero temperatures: how low can a hibernator go?. *Physiol. Biochem. Zool.* 88, 81–89. doi: 10.1086/679591

Sagot, I., and Laporte, D. (2019). The cell biology of quiescent yeast – a diversity of individual scenarios. *J. Cell Sci.* 132:jcs213025. doi: 10.1242/jcs.213025

Singhal, N. S., Bai, M., Lee, E. M., Luo, S., Cook, K. R., and Ma, D. K. (2020). Cytoprotection by a naturally occurring variant of ATP5G1 in Arctic ground squirrel neural progenitor cells. *Elife* 9:e55578. doi: 10.7554/eLife.55578

Sirois, I., Aguilar-Mahecha, A., Lafleur, J., Fowler, E., Vu, V., Scriver, M., et al. (2019). A Unique Morphological Phenotype in Chemoresistant Triple-Negative Breast Cancer Reveals Metabolic Reprogramming and PLIN4 Expression as a Molecular Vulnerability. *Mol. Cancer Res.* 17, 2492–2507. doi: 10.1158/1541-7786.MCR-19-0264

Storey, K. B., and Storey, J. M. (2012). Aestivation: signaling and hypometabolism. *J. Exp. Biol.* 215, 1425–1433. doi: 10.1242/jeb.054403

Sundaram, M., Guernsey, D. L., Rajaraman, M. M., and Rajaraman, R. (2004). Neosis: a Novel Type of Cell Division in Cancer. *Cancer Biol. Ther.* 3, 207–218. doi: 10.4161/cbt.3.2.663

Suresh, H. G., da Silveira Dos Santos, A. X., Kukulski, W., Tyedmers, J., Riezman, H., Bukau, B., et al. (2015). Prolonged starvation drives reversible sequestration of lipid biosynthetic enzymes and organelle reorganization in Saccharomyces cerevisiae. *Mol. Biol. Cell* 26, 1601–1615. doi: 10.1091/mbc.E14-11-1559

Tjaden, B. (2015). De novo assembly of bacterial transcriptomes from RNA-seq data. *Genome Biol.* 16:1. doi: 10.1186/s13059-014-0572-2

Tougeron, K. (2019). Diapause research in insects: historical review and recent work perspectives. *Entomol. Exp. Appl.* 167, 27–36. doi: 10.1111/eea.12753

Valcourt, J. R., Lemons, J. M. S., Haley, E. M., Kojima, M., Demuren, O. O., and Coller, H. A. (2012). Staying alive. *Cell Cycle* 11, 1680–1696. doi: 10.4161/cc.19879

Virchow, R. (1860). "Cellular Pathology as based upon physiological and pathological histology. Twenty lectures delivered in . 1858,"in *Translated from the second edition of the original by F. Chance. With notes and numerous emendations principally from MS. notes of the author, and illustrated by . engravings on wood*. (London: John Churchill, MDCCCLX).

Warburg, O. H. (1930). *The Metabolism of Tumours: Investigations from the Kaiser Wilhelm Institute for Biology, Berlin-Dahlem*. London: Constable & Co. Ltd.

Wilkinson, A., Hloch, A., Mueller-Paul, J., and Huber, L. (2017). The effect of brumation on memory retention. *Sci. Rep.* 7:40079. doi: 10.1038/srep40079

Wloch-Salamon, D. M., Fisher, R. M., and Regenberg, B. (2017). Division of labour in the yeast: *Saccharomyces cerevisiae*. *Yeast* 34, 399–406. doi: 10.1002/yea.3241

Yao, G. (2014). Modelling mammalian cellular quiescence. *Interface Focus* 4:20130074. doi: 10.1098/rsfs.2013.0074

Yeh, A. C., and Ramaswamy, S. (2015). Mechanisms of Cancer Cell Dormancy—Another Hallmark of Cancer?. *Cancer Res.* 75, 5014–5022. doi: 10.1158/0008-5472.CAN-15-1370

Zhang, L., Wu, C., and Hoffman, R. M. (2015). Prostate Cancer Heterogeneous High-Metastatic Multi-Organ-Colonizing Chemo-Resistant Variants Selected by Serial Metastatic Passage in Nude Mice Are Highly Enriched for Multinucleate Giant Cells. *PLoS One* 10:e0140721. doi: 10.1371/journal.pone.0140721

Zhang, S., Mercado-Uribe, I., Hanash, S., and Liu, J. (2013). iTRAQ-Based Proteomic Analysis of Polyploid Giant Cancer Cells and Budding Progeny Cells Reveals Several Distinct Pathways for Ovarian Cancer Development. *PLoS One* 8:e80120. doi: 10.1371/journal.pone.0080120

Zhang, S., Mercado-Uribe, I., Xing, Z., Sun, B., Kuang, J., and Liu, J. (2014). Generation of cancer stem-like cells through the formation of polyploid giant cancer cells. *Oncogene* 33, 116–128. doi: 10.1038/onc.2013.96

**Conflict of Interest:** KP is a consultant to Cue Biopharma, Inc., a founder and an equity holder in Keystone Biopharma, Inc., and an equity holder in PEEL therapeutics. SA is also an equity holder in Keystone Biopharma Inc.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 08 July 2021
doi: 10.3389/fevo.2021.678533

# Viewing Cancer Through the Lens of Corruption: Using Behavioral Ecology to Understand Cancer

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All biological systems depend on signals for coordination: signals which pass information among agents that run the gamut from cells to organisms. However, their very importance makes signals vulnerable to subversion. How can a receiver know whether a signal is honest or deceptive? In other words, are signals necessarily a reliable indicator of agent quality or need? By drawing parallels to ecological phenomena ranging from begging by nestlings to social insects, we investigate the role of signal degradation in cancer. We thus think of cancer as a form of corruption, in which cells command huge resource investment through relatively cheap signals, just as relatively small bribes can leverage large profits. We discuss various mechanisms which prevent deceptive signaling in the natural world and within tissues. We show how cancers evolve ways to escape these controls and relate these back to evasion mechanisms in ecology. We next introduce two related concepts, co-option and collusion, and show how they play critical roles in ecology and cancer. Drawing on public policy, we propose new approaches to view treatment based on taxation, changing the incentive structure, and the recognition of corrupted signaling networks.

**Keywords: signaling, corruption, deception, cancer, evolution, behavioral ecology, targeted therapy**

OPEN ACCESS

***Edited by:***
*Etienne Baratchart, Lund University, Sweden*

***Reviewed by:***
*Robert Noble, City University of London, United Kingdom*
*James DeGregori, University of Colorado, United States*

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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *09 March 2021*
***Accepted:*** *16 June 2021*
***Published:*** *08 July 2021*

***Citation:***
*Bukkuri A and Adler FR (2021) Viewing Cancer Through the Lens of Corruption: Using Behavioral Ecology to Understand Cancer. Front. Ecol. Evol. 9:678533. doi: 10.3389/fevo.2021.678533*

## 1. INTRODUCTION

In an influential 1996 speech, the President of World Bank, James Wolfensohn, described corruption as a cancer standing in the way of equitable development (Wolfensohn, 2005). Without giving any biological details, he used the term "cancer" to stand in generically for a bad thing that will expand if uncontrolled and is difficult to root out. In response, he prescribed what can be seen as an international version of precision medicine: specific homegrown solutions for each country, complemented by support from the World Bank for anticorruption fighters and withdrawal of international support from corrupt governments.

In economic contexts, corruption is often defined as "the abuse of public office for private gain" (Wedel, 2012), with the focus on the use of government offices with control of a limited resource to demand bribes or tariffs from a second party (Levin and Tsirik, 1998) but can be extended to include corruption such as embezzlement involving only a single individual (Boisvert et al., 2014). Mathematical analysis began with Rose-Ackerman (Rose-Ackerman, 1975) who studied bribes using game theory and emphasized the role of information availability and reliability. Despite some special cases where corruption might "grease the wheels of commerce" by correcting distorted

markets, the effects of corruption are widely agreed to be destructive (Wei, 1999), as argued by Wolfensohn. Corruption concentrates wealth and power in the hands of those with access, reducing resources for the environment and the poor (Joly, 2017).

The causes of corruption are perhaps as complex as those of cancer, involving internal forces that parallel growth and mutation, outside influences like colonialism that parallel carcinogens or oncogenic viruses, and a history that parallels how these forces develop in a tumor (Wedel, 2012). Incompetence can create supply gaps to be filled by corruption, excessive or ambiguous regulation might promote their evasion, recessions can increase need and increase incentives for corruption, and low pay or education of government officials might increase temptation (Levin and Tsirik, 1998). Corruption occurs within a complex social system, just as cancer occurs within the tightly knit environment of a tissue, and is promoted by a lack of transparency and accountability (Levin and Tsirik, 1998), but also by the very social pressures that make societies function (Kolokoltsov and Malafeyev, 2017). Once established, corruption can spread like a contagion (Nekovee and Pinto, 2019) or, of course, like a cancer.

Direct approaches to combating corruption address these causes by promoting greater transparency or increasing pay and training (Levin and Tsirik, 1998). More systemic approaches look to institutions. The benefit of modular network structures that can contain corruption in more isolated cells must be balanced against the risk that isolated units could receive less oversight (Luna-Pla and Nicolás-Carlock, 2020). In some cases, disrupting the structure of established corruption networks might even improve their functioning (Duijn et al., 2014). Fighting corruption, like fighting cancer, must thus deal with the Law of Unintended Consequences (Fisman and Golden, 2017). As anyone who watches crime movies knows, the most difficult corruption to detect and control goes all the way to the top, a modern variant of the ancient question "But who will guard the guardians?" (Hurwicz, 2007).

Here, we ask whether the metaphor of corruption as a cancer can be inverted, and whether thinking of cancer as corruption might provide new approaches to treatment. This thinking seeks to extend the many ideas derived from cancer ecology (Pienta et al., 2008; Gatenby et al., 2009; Korolev et al., 2014; Kareva, 2015) to a behavioral ecology framework that sees cancer as a breakdown of the reliability of signaling and information transfer needed to coordinate complex biological systems.

## 2. COMMUNICATION AND SIGNALING IN BIOLOGICAL SYSTEMS

Coordination of complex biological systems, from cells and tissues to societies, depends on reliable communication. This communication underlies behaviors ranging from the intricate intracellular signaling cascades that coordinate cell growth, movement, and division (Alberts et al., 2014) to the "ballerina dances" that male birds-of-paradise use to court females (Wilts et al., 2014). Communication requires signals, pieces of information transmitted from a signaler to a receiver (**Table 1** provides precise definitions of key terms used throughout this paper). Signals can be chemical (cytokines in the immune system), aural (begging calls of nestlings), or visual (skin coloring of venomous snakes or body movements of courting birds). Following (Otte, 1974), we define a signal as a trait that plays an adaptive role through conveying information to other organisms. Traits like body size do convey information, but we treat as signals only when modified to alter perception through structures like frills (Shine, 1990). We discuss the blurry line between physical constraints and signals later.

**TABLE 1 |** Glossary of key terms.

- **Condition-dependent handicap:** Low quality individuals must pay a greater price to signal than high quality individuals
- **Condition-dependent payoff:** High need individuals receive a greater benefit from signaling than low need individuals
- **Corruption:** Abuse of public office for private gain, or more broadly, a violation of the public trust
- **Deception:** A difference in the receiver's interpretation of a signal, upon which it acts, and the state of the signaler
- **Information:** Stimulus that has meaning in some context for its receiver, here typically the need, quality, or state of an individual
- **Signal:** A medium that transfers information from one individual (the signaler) to another (the receiver)

Communication depends on the response of the signal receiver. When a signal is reliable, the receiver can accurately ascertain information about the state of the signaler. Drawing upon (Searcy and Nowicki, 2005), we classify a signal as reliable if: (1) a characteristic of the signal is consistently correlated with some attribute of the signaler or environment and (2) if receivers gain some benefit from having information about the attribute. For instance, if the call of a male frog is consistently correlated with its size and if knowing the size of male frogs allows females to choose appropriate mates, the male frog call is reliable. We define deception functionally, obviating the need to assume cognitive underpinnings for deceptive behavior (Hauser, 1996). Again, following (Searcy and Nowicki, 2005), we define deception as when (1) a receiver registers Y from a signaler, (2) the receiver responds in a manner that benefits the signaler and is appropriate if Y means X, but (3) it is not true that X is the case. For example, in great tits (Møller, 2010) and many other birds, alarm calls are sometimes given in the absence of predators to induce competitors to move away from a food source.

More broadly, deception can be categorized into three classes: exaggeration (use of a signal that differs from the corresponding condition), lies (use of the wrong signal), or withholding information (not signaling when appropriate) (Vehrencamp, 2009). Each of these is context-dependent. For example, if every frog were to sing as if it were larger than its true size, receivers could adjust their interpretation to accurately assess them, canceling the effects of exaggeration and making deception a function of the social environment. In this context, we think of corruption as being of the network of communication itself, such as when a government official conceals information to demand bribes for access. One can think of counterfeit money as a deceptive signal of value that corrupts the trust in the "legal

tender" upon which the economy depends. Before taking on the broader concept of corruption, we focus on the mechanisms by which deception is deterred in ecology and in tissues. We then describe how cancers can evolve to evade these checks and relate these strategies to those used in ecology. Finally, we describe how viewing cancer through the lens of corruption, combined with appropriate mathematical models, can inspire new treatment strategies.

## 3. DECEPTION DETERRENCE

Deception degrades the reliability of the communication system, endangering the coordination on which complex systems depend. The fable of The Boy Who Cried Wolf provides a proverbial example. However, conflicts of interest between signallers and receivers can favor degrading reliability, as with alarm calls. How then can biological systems maintain the integrity of communication (Searcy and Nowicki, 2005; Vehrencamp, 2009)?

We review key mechanisms from behavioral ecology and discuss their application to tissues. First, and perhaps most obvious, biological systems can reduce or remove conflicts of interest (Krakauer and Pagel, 1996). The high relatedness of sterile worker ants within a colony and the segregation of germ and somatic tissue play similar roles: individual ants or cells gain no benefit from behaviors that fail to aid the centralized reproduction of the collective. However, the ability of many worker ants to lay unfertilized male eggs creates such a conflict, creating a challenge for colony functioning (Monnin and Ratnieks, 2001). Avoiding such genetic conflicts within the nest favors loss of kin recognition and the use of colony odor to distinguish nestmates from non-nestmates (Lenoir et al., 2001). To avoid attack, workers emerge with minimal odor, and are tolerated by older workers while they come to smell like their colony. Slave-making ants use this necessary acceptance of ambiguous workers to take over a colony. A queen from a slave-making species can infiltrate a host colony, kill the existing queen, and lay her own eggs to be taken care of by the accepting workers (Buschinger, 2009).

In evolutionary ecology, costly signaling is perhaps the best-known deterrence mechanism; a signal constrained in this way is called a handicap signal (Vehrencamp, 2000). With condition-dependent handicaps, low quality individuals pay a greater cost to signal than high quality individuals. If the benefit for a given signal intensity is identical for all signalers, the optimal signaling level that maximizes the difference between signal benefit and cost will be greater for high quality individuals (**Figure 1**) (Grafen, 1990).

Ecologists have established many cases of this mechanism. Territorial song by male birds is an energetically expensive and time-consuming signal of physical state and territory quality. Males with higher quality territories have more access to food, and thus pay a lower cost to sing more frequently because they need to dedicate less time to foraging. Experiments across bird species have confirmed this prediction by modifying food availability in male territories and quantifying changes in song frequency (Hoi-Leitner et al., 1995; Manica et al., 2014). For females, this signal then conveys reliable information about which males can provide the best access to food and potentially greater investment in offspring care (Rytkonen et al., 1997). Signals play a key role in the other component of sexual selection, male-male competition. In red deer, energetically costly roaring contests use muscles and actions similar to those used in fighting and thus provide reliable information to assess male fighting condition (Clutton-Brock and Albon, 1979). In our own bodies, cells may also use costly signals to display their phenotypic state to other cells in competitive environments. During development of the neural system and of eggs, for example, costly signals identify the healthiest cells that should be maintained for the good of the body despite the lack of a conflict of interest (Krakauer and Pagel, 1996; Madan et al., 2018).

Costly signals of need follow a similar logic if the greatest benefits accrue to those with the greatest need. If all signalers pay the same cost to signal but differ in their benefit curves, we expect the signaler with the higher benefit curve to maximize fitness by signaling at a higher intensity (**Figure 2**). Birds provide the well-studied example of begging by nestlings. A starving nestling should receive a greater benefit from a morsel of food than a well-fed nestling and will thus beg more intensely from its parents who receive a reliable signal of need. Experimental manipulation of hunger by artificial feeding or short-term food deprivation in a variety of bird species has shown that begging reliably signals short-term need (Sacchi, 2002; Watson and Ritchison, 2018), with birds that were deprived of food begging at significantly higher rates than those that were not (Cotton et al., 1996; Kilner et al., 1999). In our cells, a similar low benefit mechanism may help suppress uncontrolled cell proliferation. Cells require growth factors to divide, and can signal for these dependent on need. The larger array of internal controls, including cell cycle checkpoints (Kastan and Bartek, 2004; Barnum and O'Connell, 2014), programmed cell death (Elmore, 2007; Fuchs and Steller, 2011), and oncogene-induced senescence (Gorgoulis and Halazonetis, 2010; Zhu et al., 2020) mean that the effects of growth factors saturate. Like a fully fed nestling, a cell that takes up massive amounts of growth factor would grow no faster, removing selection for deceptive signaling.

These cost-benefit mechanisms are complemented by enforcement. We thus distinguish the energetic or production costs characteristic of condition-dependent handicaps from externally imposed costs through punishment or retaliation. With repeated interactions, if receivers can identify and remember the sources of deceptive signals, they can discriminate against them in the future and reduce or reverse the benefits of deception (Searcy and Nowicki, 2005). This individually directed skepticism can enforce signal reliability because the benefit a signaler receives from one deceptive interaction can be outweighed by the cost of lower receiver responses in the future (Maynard-Smith, 1991; Silk et al., 2000). As we have seen, some animals use false alarm calls to lure competitors away from food. In an experiment with Richardson's ground squirrels, when the alarm calls of one squirrel were consistently paired with the approach of a badger and the calls of another squirrel were not, receiver squirrels displayed much higher

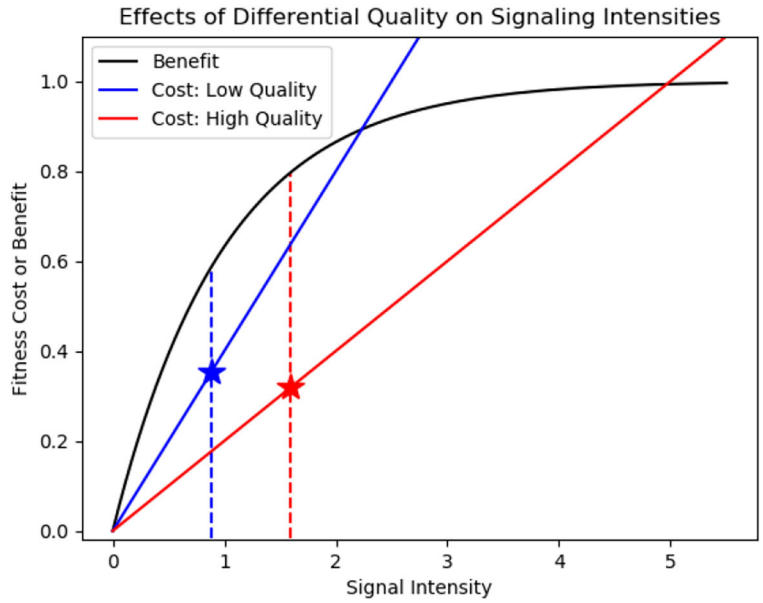


**FIGURE 1 |** Lower signaling costs support higher signal intensities for high quality signalers (inspired by Johnstone, 1977). The black curve represents the fitness benefit of signaling for a signaler. The blue curve represents the fitness cost of signaling for a low quality signaler: the cost of signaling increases rapidly with signal intensity. The red curve represents the fitness cost of signaling for a high quality signaler: the cost of signaling increases more gradually with signal intensity. Signalers will signal at an intensity that maximizes the difference between benefits and costs (indicated by blue and red stars). Because the cost curve is less steep for high quality signalers, the intersection point will occur at a higher signal intensity.

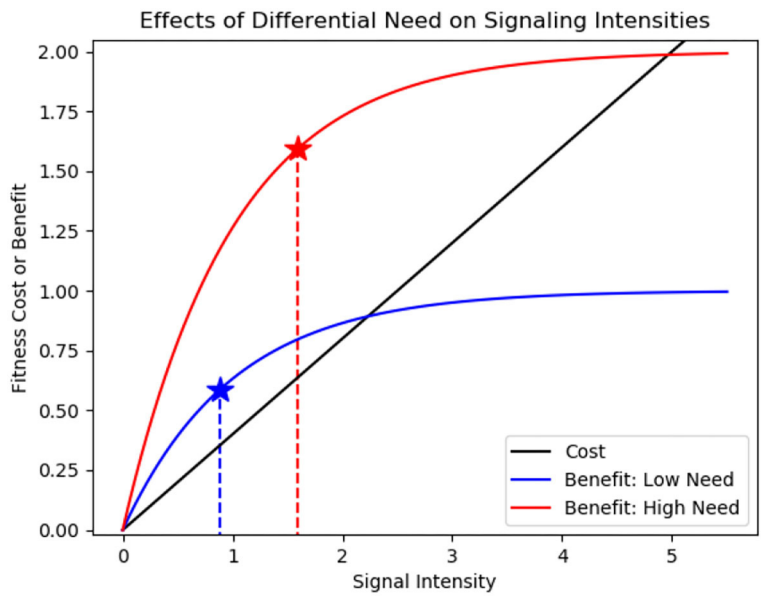


**FIGURE 2 |** Higher signaling benefits support higher signal intensities for needy signalers (inspired by Johnstone, 1977). The black curve represents the fitness cost of signaling for signalers. The blue curve represents the fitness benefit of signaling for a low need signaler: the benefits of signaling quickly saturate with signal intensity. The red curve represents the fitness benefit of signaling for a high need signaler: the benefit of signaling saturates more gradually with signal intensity. Signalers will signal at an intensity that maximizes the difference between cost and benefits (indicated by blue and red stars). Because the benefit curve saturates less quickly for high need signalers, the intersection point will occur at a higher signal intensity.

levels of vigilance to the alarms of the honest squirrel than to the dishonest one (Hare and Atkins, 2001). Similar results have been seen in vervet monkeys (Cheney and Seyfarth, 1988) and food calls in domestic chickens (Evans and Evans, 2002). One could speculate that the lack of alarm calls in the gregarious zebra finch could be the endpoint of this breakdown, where

**TABLE 2 |** Mechansisms of maintaining signal reliability in ecology and in tissues.

| Prevention mechanism | Ecological example | Tissue homeostasis |
|---|---|---|
| Eliminate conflict of interest | High relatedness | Segregation of germ plasm |
| Signal costs | Song output | Signaling complexity |
| Signal benefits | Begging | Checkpoint controls |
| Enforcement | Testing through conflict | Immunological surveillance |
| Physical constraints | Vocalization frequency | PAMPs and TLRs |

birds have abandoned such calls to be replaced by visual cues (Butler et al., 2017).

Instead of removing the benefit of deception, receivers could directly retaliate against deceptive signalers. For example, the highly variable black clypeus patterns on the female paper wasp *Polistes dominulus* strongly predict body size and social dominance (Tibbetts and Dale, 2004). Specifically, wasps with no marks or single marks are less aggressive and defer to the more aggressive wasps that have "broken" facial patterns (Clark and Kimbrough, 2017). However, the reliability of this signal is constantly tested in contests between wasps. Experimental results show that wasps with experimentally altered clypeus patterns (deceptive signalers) received considerbly more aggression from rivals and were less able to establish dominance relationships, suggesting that a mismatch between signal and state causes social punishment (Tibbetts and Dale, 2004; Tibbetts and Izzo, 2010). The constant testing of host tissues for self-antigens such as MHC and proteins like decay-accelerating factor that regulates the complement system quickly identifies any cells that fail to provide appropriate signals. Immunological memory serves as a form of repeated interaction. When a tissue is exposed to an antigen on several occasions, the host produces memory B and T cells that enable a more rapid and effective adaptive immune response to subsequent antigen insults (Janeway et al., 2001).

Most simply, physical constraints can prevent deceptive signaling (Vehrencamp, 2000) if signal intensity is tightly correlated with the quality being signaled, and cannot be faked (Maynard-Smith and Harper, 2003). For example, the frequency of vocalization depends on the size of vocal folds in vertebrates, which in turn depends on body size. This size-frequency allometry has been experimentally confirmed across many mammalian species, from primates to carnivores (Morton, 1977; Schmidt-Nielsen, 1984; Bowling et al., 2017). If body size is a marker for male quality, females can use vocalization frequency signals to choose the best mate (Glaudas et al., 2020). Similarly, song repertoire size in great reed warblers is an index of age, with older males having a greater repertoire size (Hasselquist, 1994). However, no correlation is perfect, and any trait that allows a signaler to sound larger without being larger would be favored, leading to a shift in the whole signal. In the context of tissue homeostasis, pathogens may carry signals (pathogen-associated molecular patterns or PAMPs) as seemingly essential parts of their physical structure, such as LPS in the cell wall of gram negative bacteria or a production of double-stranded RNA during replication of most RNA viruses (Maverakis et al., 2015). These PAMPS are reliably recognized by innate mechanisms of the immune system, such as toll-like receptors (TLRs), triggering an immune response. (Kumar et al., 2011). Of course, pathogens have no interest in signaling their presence, and have evolved many ways to modify or conceal these signals. Similarly, as we will see, cancer cells may modify their cell surface markers to hide from immune cells. More simply, pathogens, by definition, damage their hosts and this physical damage creates an unambiguous signal. A summary of these prevention mechanisms and examples in ecology and tissue homeostasis is captured in **Table 2**.

## 4. ORIGINS OF CORRUPTION AND THE MAINTENANCE OF CANCER

When signaling systems include conflicts of interest between signalers and receivers, the reliability of communication is in constant flux. Cancers emerge by corrupting these control measures perhaps first and foremost by creating a conflict of interest within a tissue. Even when control begins to unravel, as seems inevitable with aging (Martincorena et al., 2015), mechanisms based on costs, benefits, repeated interactions, enforcement, and constraints can maintain effective communication. But breakdown of the systems through systematic deception or evasion is always possible. In this section, we outline the ways cancers have evolved to partially or completely circumvent each of these deterrence mechanisms, and relate these evasion strategies back to ecology.

We begin by proposing that evasion of high cost and low benefit mechanisms is central to the rise of corruption in cancer. For costs, we propose that there are three main types: internal regulation, external enforcement, and energetic costs. Internal regulation refers to internal controls that cells have to govern proliferation such as cell-cycle checkpoints and oncogene-induced senescence. External enforcement describes interactions of cancer cells with other agents in their microenvironment, most notably immune cells. Energetic costs refer to the actual costs cells incur to grow and divide, including ATP, pathway intermediates, and synthesis of critical molecules. We here focus on the energetic costs of cells and consider internal regulation and external enforcement later. Assume there are two agents identical in quality, but differing in the cost of signaling (**Figure 1**). The individual with a lower signaling cost would be expected to signal at higher intensities and benefit from this cost differential in a deceptive fashion. Similarly, if one individual receives a higher benefit from signaling, we expect this individual to signal at

a higher intensity (Johnstone, 1977; Grafen, 1990). In cancer, we hypothesize that both of these cases occur, resulting in an "exaggeration" evasion mechanism.

In order for a cell to divide or secrete growth factors for example, the coordination and contribution of a multitude of signaling components are needed. These components are tightly regulated through both environmental and intracellular factors to ensure that the cell does not divide at an inappropriate time. However, in cancer, due to the presence of oncogenes, these signaling components can be overexpressed or constitutively active, removing the need to meet certain intracellular or extracellular conditions and leading to uncontrolled levels of proliferation. For example, MYC codes for c-Myc, which induces cellular proliferation (Dang, 2012; Tansey, 2014). Normally, it becomes activated upon receiving mitogenic signals like serum stimulation, Wnt, Shh, or EGF via the MAPK/ERK pathway (Campisi et al., 1984). However, in many cancers, most notably in Burkitt's lymphoma, Myc is constitutively expressed. This removes the need for the cells to receive external serum stimulation or expend energy to produce these signals, leading to increased expression of many downstream genes that govern cell proliferation (Finver et al., 1988). Similar situations occur with NF$\kappa$B and STAT3, key players in cell proliferation, apoptosis, migration, and angiogenesis. Normally, these are activated by a plethora of cytokines and growth factors; however, due to constitutive activation in cancer, the cells do not need to invest in the production of these cytokines and growth factors to activate them, reducing the cost of proliferation, movement, and blood vessel recruitment (Garcia et al., 2001; Nagel et al., 2014). More generally, cancer cells seem to operate with a greater degree of modularity; rather than having tightly integrated gene signaling networks, pathways may act more independently of each other, and thus avoiding some of the energetic costs involved in growth and division.

Quite generally, cancers escape by removing the controls that create saturating benefits. When cells lose tumor suppressors such as Rb, they can grow more quickly for a given amount of nutrient or growth factor, while a normal cell would be prevented from dividing too quickly. Cancer cells that acquire mutations to signal at higher intensities would then grow more quickly, creating selection for deception (Vehrencamp, 2009). One can see these cancer cells as exaggerating their phenotypic state and need for growth and division factors. Cancer cells can also evade saturating benefits by modifying their metabolic pathways. Most cancers exhibit the Warburg effect, the use of glycolysis rather than oxidative phosphorylation to generate ATP (Liberti and Locasale, 2016). Although glycolysis is less efficient at generating ATP, it creates more intermediates for biosynthetic and anabolic purposes (e.g., through the pentose phosphate pathway) and greater metabolic flexibility (e.g., catabolism of macromolecules) when nutrients are limited (Vander Heiden et al., 2009). This production of proteins, amino acids, and lipids is crucial for proliferation. Unlike normal cells whose proliferation is partially constrained, intrinsically and extrinsically, by the rate at which they can produce molecules needed for growth and division, cancer cells can rapidly produce those needed molecules. One example of subversion of enforcement of honesty through the saturating benefit mechanism in ecology, cowbirds lay their eggs in the nests of other often smaller birds, and continue to beg and grow far beyond their hapless nestmates (Dearborn, 1998).

Even the most tightly knit societies, such as social insects, have interaction intensity vastly exceeded by that of cells in a body. Due to the huge number of cells in the body or in a tumor, individual cells are not encountered multiple times and remembered. However, the whole tumor does present novel antigens in the context of damage, triggering an immune response. Cancers capitalize on one essential feature of this response, T cell exhaustion. If the immune system indefinitely attacked every repeated challenge, autoimmunity would be almost unavoidable in the face of low level inflammation. When faced with slow-growing tumors, immune cells interact repeatedly with antigens on the surface of cancer cells, promoting the progressive loss of function of effector T cells (Wherry, 2011; Schietinger and Greenberg, 2014). In addition, when chronically exposed to antigen, tumor-specific T cells develop an increased expression of many inhibitory receptors such as PD-1 and CTLA-4 and an altered cellular metabolic and transcriptomic profile. As a result, these T cells have lowered proliferation, effector cytokine secretion, and cytolytic activity, aiding tumor-immune escape (Jiang et al., 2015; Catakovic et al., 2017; Zhang et al., 2020). Tumor cells amplify these natural controls in at least two ways, first by reducing expression of class I MHC (Vinay et al., 2015) (a form of deception through withholding information) and through production or induction of production of immune suppressing signals like PDL1 (Cha et al., 2019). As described earlier, slave-making ants and certain beetles capitalize on the tolerance workers show to bland-smelling intruders (Geiselhardt et al., 2007).

Finally, cancers find ways to evade even the physical constraints that prevent deception. Normally, immune cells circulate through the body, detect, and destroy malignant or premalignant cells in a process called immune surveillance (Swann and Smyth, 2007). They do so by identifying tumor-specific antigens that are present on a cancer cell's surface, which triggers killing of the cancer cell through such mechanisms as release of cytotoxic molecules like granzymes and perforin (Tsukumo and Yasutomo, 2018). Tumors use many signal corrupting mechanisms to avoid the immune system, including the release of immunosuppressive cytokines (Seliger, 2005) and recruiting regulatory T cell function (Zindl and Chaplin, 2010). Cancer cells can also mask their identity through the modification of antigen presentation (Vinay et al., 2015). In particular, cancer cells can downregulate MHCI expression (the molecule used to present antigens to the immune system) or lack the requisite costimulatory molecules for antigen presentation. In this way, the cancer cell becomes invisible to the T cells, which cannot recognize it as "non-self."

Cancer cells can also gain a competitive advantage over neighboring cells by modifying their expression of certain membrane proteins. Cell selection based on "fitness fingerprints" is used in development and maintenance to identify and eliminate cells with low fitness relative to their neighboring cells (Madan et al., 2018). The membrane bound protein Flower has different isoforms termed "Win" and "Lose" to signal cell

**TABLE 3 |** Mechanisms of evasion in ecology and cancer.

| Prevention mechanism | Evasion mechanism | Ecological example | Cancer example |
|---|---|---|---|
| Eliminate conflict of interest | Mutation | Worker reproduction | Uncontrolled growth |
| Signal costs | Exaggeration | Cowbird begging | Oncogenes |
| Signal benefits | Exaggeration | Cowbird begging | Loss of tumor suppressors |
| Enforcement | Withholding information | Social insects | T cell exhaustion |
| Physical constraints | Lies | Flock foraging | Immune-evasion markers |

quality. Cells that express high levels of Lose isoforms are marked as low fitness and, if surrounded by cells expressing high levels of Win isoforms, are eliminated (Madan et al., 2019). This process allows the body to delay aging (Merino et al., 2015), prevent developmental malformation (Merino et al., 2013, 2015), and replace old tissues during regeneration (Moreno et al., 2015). However, this cell selection mechanism can be hijacked by premalignant cancer cells, which upregulate Win isoform expression regardless of cell quality, to gain a competitive advantage at the expense of neighboring stromal cells that express the Lose isoform, increasing the cancer cell's proliferative and metastatic potential (Madan et al., 2019).

Cancer cells avoid a different physical constraint through the loss of contact inhibition. Non-cancerous cells initiate cell cycle arrest and reduce proliferation and mitogen signaling pathways when cellular density is too high and cells are in contact with each other, regardless of their cellular metabolism or extracellular factors (Levine et al., 1965). However, this response is weakened in cancer cells, allowing them to proliferate uncontrollably and grow on top of each other, leading to the high density characteristic of solid tumors (Pavel et al., 2018). We summarize of the evasion strategies associated with each of these prevention mechanisms along with examples in ecology and cancer in **Table 3**.

## 5. COUSINS OF CORRUPTION

Two related concepts enhance the danger of cancer corruption: co-option and collusion. co-option means diverting resources or assistance in roles different from usual, and instead adopting them for one's own sake. Cancer cells can co-opt normal cells in the tumor microenvironment to work for them. Viewing cancer as "the wound that never heals" (Hua and Bergers, 2019) reveals this process: the chronic inflammation that often accompanies cancer progression brings with it a variety of inflammatory agents which can lead to the infiltration and activation of different myeloid cells such as macrophages that contribute to growth (Schmid and Varner, 2012; Stegelmeier et al., 2019). Thus, the physical damage that should be an unambiguous signal of danger is not concealed but is instead turned to the tumor's own advantage. As part of this process, some cancer cells release cytokines that promote polarization of nearby immune cells to a pro-tumor role, dampening the anti-tumor immune response and stimulating cancer cell survival and proliferation (Cheng et al., 2019; Strauss et al., 2020). Immune cells can promote cancer progression through an "angiogenic switch" by producing proteases, proteins that break down the extracellular matrix, that in turn can activate latent molecules to drive angiogenesis (Ribatti et al., 2007; Baeriswyl and Christofori, 2009). Furthermore, recent evidence in the context of breast cancer suggests that metastatic cancer cells can induce regression of normal non-cancerous cells in their local environment into a stem-like state, further promoting tumorigenesis (Ombrato et al., 2019). In ecology, some forms of sexual selection co-opt prior preferences, such as those of female birds for brightly-colored fruit, to create attractive males (Ryan et al., 1990). As we have seen, brood parasitism provides an example of how one species co-opts another. Brood parasites like cuckoos and cowbirds manipulate a host to care for their offspring, leaving them with time and energy to spend on feeding and producing more offspring (Dearborn, 1998). Egg-dumping is common within species, where individuals again co-opt the parental care instincts of others (Yom-Tov, 1980).

When corruption becomes systemic, multiple individuals can work together in complementary roles and collude to garner resources and subvert the signaling environment. Cancer cells, both within and among tumors, can "collude" by exchanging information, such as RNA, DNA, and proteins, through exosomes and other mechanisms (Li et al., 2006; Hough et al., 2017; Maziveyi et al., 2019). Although far from fully understood, the proteins, metabolites, and nucleic acids delivered in this way are thought to facilitate survival, differentiation, and proliferation, promote angiogenesis and wound healing, contribute to metastasis and migration, and reprogram metabolic profiles of receiving cells (Kalluri and LeBleu, 2020). For example, cancer cells in hypoxic conditions secrete exosomes with increased angiogenic and metastatic potential to engineer a more favorable environment or move to a new one (Park et al., 2010). Exosomes from tumor cells mediate the metastasis of cancer to distant organs through uptake by resident cells that prepare the pre-metastatic niche (Hoshino et al., 2015). Poly-aneuploid cancer cells (PACCs) are a recently discovered form of collusion in cancer. During times of microenvironmental stress, aneuploid cancer cells can fuse together to form PACCs, entering a state of quiescence or reversible therapy-induced senescence to protect their genome and avoid apoptosis (Pienta et al., 2021). Due to their high levels of genomic content, PACCs that enter the cell cycle and divide into non-polyploid cells can produce new phenotypic variants of cancer cells that contribute to cancer heterogeneity and lethality (Bukkuri et al., under review; Bukkuri et al., under preparation).

In ecology, "collusion" is usually seen as cooperation that does not subvert the existing order, such as food sharing among

**TABLE 4 |** Hallmarks of cancer and escape mechanisms viewed through the lens of corruption.

| Hallmark of cancer | Escape mechanism |
|---|---|
| Deregulating cellular energetics | Modification of costs and benefits within cells |
| Sustaining proliferative signaling | False signals of need |
| Evading growth suppressors | Evasion of signals of control |
| Avoiding immune destruction | Evasion of enforcement: false or concealed information |
| Enabling replicative immortality | Evasion of physical constraint through telemorase |
| Tumor-promoting inflammation | Increasing access to resources through signals of need |
| Activating invasion & metastasis | Corrupting distant tissues and colluding to enhance invasion |
| Inducing angiogenesis | Exaggerated signals of need |
| Genome instability and mutation | Corruption of control systems maintaining cell integrity |
| Resisting cell death | Evasion of control signals |

vampire bats (Carter and Wilkinson, 2013). Coalitions of males working together to oust an existing leader is perhaps closer to the human sense of the term, but hardly subverts an already violent social order (De Waal and Waal, 2007). The "dear enemy" effect, where neighboring territory owners cooperate by reducing aggression (Temeles, 1994) is not a breakdown of the territorial system itself, but a modification through cooperation that can even enhance defense against intruders (Detto et al., 2010). One could view sexual reproduction as a form of genetic collusion. Similar to the mixing of genetic material in the PACC state through cell fusion that produces increased heritable variation, many asexually reproducing species engage in sexual recombination when under stress, ranging from the crustacean *Daphnia magna* that produces males and sexual eggs when facing high population density, starvation, or bacterial infection (Kleiven et al., 1992; Mitchell et al., 2004) to the perennial herb *Trifolium repens* that increases investment in sexual reproduction when subject to herbivory. As an interesting parallel with cancer, this response was observed solely in sensitive plants and not resistant ones (Griffiths and Bonser, 2013). This view of cancer as a corruption of the signaling system aligns remarkably well, although not perfectly, with the established hallmarks of cancer (Hanahan and Weinberg, 2011) (**Table 4**).

## 6. TREATMENT: CARROT OR STICK?

Does viewing cancer through the lens of signaling and corruption help us design treatments? Our goal is to re-establish the broken control system, either through some form of punishment (the stick) in parallel with strengthening enforcement mechanisms, or restoring the incentive structure created by costs and benefits of signaling so that the corrupt behavior is no longer beneficial (the carrot).

### 6.1. The Stick: Punishing Corruption

One way to abolish corruption is by punishing corrupt behavior directly, including regulation and taxation. Regulation takes many forms in the body, both within and across cells, such as the immune system detecting cancer cells by their novel antigens and destroying them. Immunotherapies, drugs that boost the immune system, can restore regulation often weakened through deceptive signals by the cancer. In this section, we focus instead on taxation. In society, taxes are a tool of the public sector to guide behavior in socially preferred ways, which has no direct parallel in the self-organized and decentralized body. We here think of taxation as having been guided by natural selection that has evolved policies to control corrupt actors much as laws and societies constantly develop and learn to achieve the same ends. In both cases, corrupt actors almost by definition are not playing by the rules, and can find ways to change their strategy to evade or subvert the strategies, as the constant effort to suppress new forms of tax fraud illustrates. However, such fraud requires altered behaviors, such as hiring clever lawyers and fixers, that create inefficiencies that parallel the costs of developing drug resistance.

We begin with two brief examples of the unintended consequences of anti-corruption efforts in public policy (Fisman and Golden, 2017). Due to rampant cheating on high school exit exams in Romania, security cameras were introduced to monitor students and teachers. Although cheating overall was reduced, the policy disproportionately impacted the poorer students because more affluent students were able to bribe the enforcers individually without having to engage in more detectable collusion like poorer students (Borcan et al., 2017). In Ghana, the placement of observers at select polling locations did succeed in reducing fraud at these locations, fraudulent activity increased in neighboring, unobserved polling locations. These examples illustrate two key points: (1) anti-corruption measures can disproportionately impact certain groups and (2) anti-corruption efforts are always under threat of subversion by corrupt actors who find a way to modify their behavior to evade them.

A related challenge arises with the use of targeted therapies to treat cancer. Targeted therapies, from monoclonal antibodies to small molecule inhibitors, have been at the forefront of the precision medicine revolution, promising effective treatments tailored to each patient's unique genetic profile. In contrast to chemotherapy that affects all rapidly dividing normal and cancerous cells, targeted therapies attack pathways specifically associated with the patient's cancer. This reduces side effects and should be more potent than standard chemotherapy. Like specific anti-corruption policies however, targeted therapies only attack a specific *form* of corruption, and cancer cells can modify their corruption strategy through mutation or plasticity to avoid effects of the drug, creating an opportunity to evade the treatment. In a simple graphical model, an untreated cancer might grow most quickly with a particular pathway tuned to an intermediate value (**Figure 3**). Chemotherapy (blue line) reduces growth of all cells below that needed for replacement (dashed gray line). Targeted therapy (red line) lowers growth of the most rapidly growing phenotype even further, but leaves a window of escape for corrupt cells.

As an example, trastuzumab is a targeted therapy for HER2+ breast cancer (Slamon et al., 2001). Despite being initially highly effective, the majority of patients still experience

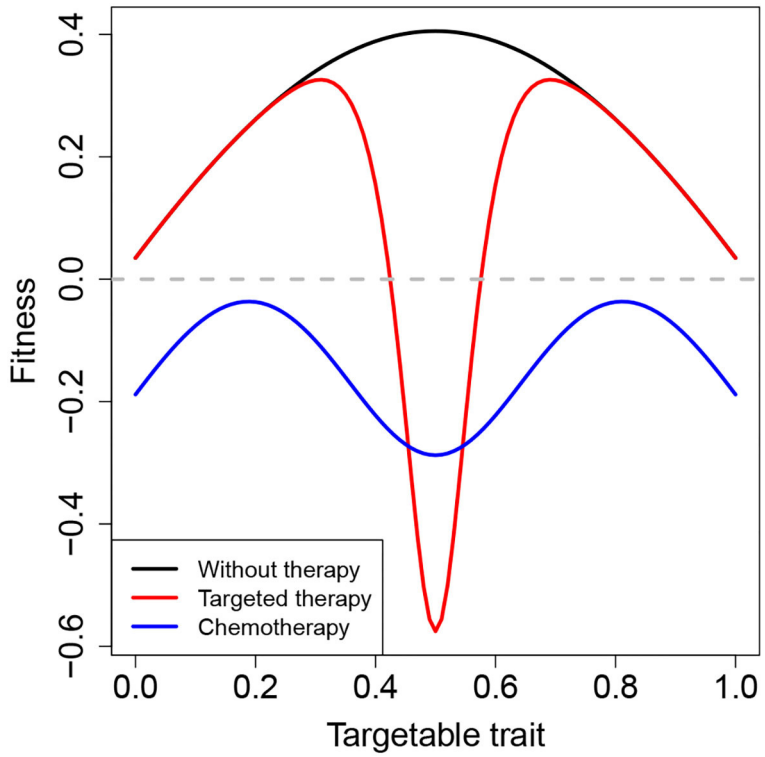


**FIGURE 3 |** Hypothetical fitness landscape in response to therapy. The black curve represents the fitness of the cancer cells in the absence of therapy, which is maximized for an intermediate value of some pathway trait. Targeted therapy, represented by the red curve, greatly diminishes the fitness of cancer cells with intermediate trait values. However, in so doing, it leaves room for cancer cells with more extreme trait values to survive. The blue curve depicts the effects of chemotherapy, which broadly reduces the fitness of all cells below a critical threshold, although not as dramatically as the targeted therapy on cells with intermediate trait values.

disease progression within 1 year (Ellis and Hicklin, 2009). This resistance emerges through a disturbingly wide array of mechanisms: (1) mutation of the HER2 target to prevent binding of the drug, (2) upregulation of downstream signaling pathways, (3) upregulation of alternate growth factor signaling pathways, and (4) inhibition of immune-mediated mechanisms (Pohlmann et al., 2009). Imatinib was one of the first targeted therapies, proving highly effective against BCR-ABL, a gene highly overexpressed in almost every case of chronic myeloid leukemia (Ellis and Hicklin, 2009). In this case, resistance could be caused by (1) amplification of the BCR-ABL target, (2) mutations in the BCR-ABL domain to prevent binding of the drug, and (3) the emergence of BCR-ABL independent pathways for signal transduction (Milojkovic and Apperley, 2009). Although targeted therapies can more efficiently kill cancer cells while sparing healthy cells, they disproportionately affect sensitive cells and provide opportunities for evolution of new strategies that bypass the drug's target molecule. Traditional chemotherapy which simply targets rapidly dividing cells may thus be more effective at keeping up with the cancer cell's evolution, recalling Haldane's wonderful remark "It is much easier for a mouse to get a set of genes which enable it to resist *Bacillus typhimurium* than a set which enable it to resist cats" (Lederberg, 1999).

In this context, we propose viewing cancer treatment as a form of taxation. In order for cells to continue to survive and proliferate under treatment, they must pay some cost, or tax, by developing a mechanism of drug resistance. This can take forms that include spending energy to upregulate production of the target molecule or utilizing a less energetically efficient signaling pathway to grow and divide. In economic terms, targeted therapies act on *elastic goods*, goods that can be easily replaced by alternatives if prices rise. Because targeted therapies focus on one small aspect of complex, multi-agent signaling pathways, cancer cells can evade the tax by shifting to an alternative. In contrast, standard chemotherapies affect all rapidly dividing cells, agnostic of the specific form of corruption of cancer cells. Thus, cancer cells are left with the option of dividing less, exactly as we hope, or mutating to defend against the drug's effects, such as through drug anti-porters (Lage et al., 1999; Tawbi and Buch, 2010; Jiang et al., 2011), defective apoptotic pathways (Bedikian et al., 2006), or the upregulation of survival signals (Lev et al., 2003). To continue our economic analogy, chemotherapy acts on *inelastic goods*, such as gasoline, whose consumption does not change much as a function of price. Rather than switching to an alternate pathway, standard chemotherapy forces cells to pay a price to continue using the same pathways for growth and division. A narrowly targeted tax is more effective when it works, but is easier

to evade, while a broad tax affects the whole economy but is more difficult to avoid. With cancer, these arguments for and against traditional chemotherapy or targeted therapies depend on how quickly the cancer can evolve or alter behavior to escape treatment (Bukkuri et al., under review).

### 6.2. The Carrot: Changing Incentives

An alternative approach to prevent corruption changes the incentive structure to remove the benefits of corrupt behavior. In cancer, there are many ways to change the incentive structure to reduce the benefit of rapid proliferation. We discuss three approaches: oncogene-induced senescence (OIS), traditional chemotherapy, and the sucker's gambit. Although OIS can play both pro- and anti-tumor roles (Gorgoulis and Halazonetis, 2010; Liu et al., 2018), we focus on its role in suppressing excessive cell proliferation by arresting the cell cycle upon recognition of aberrant oncogenic signaling (Zhu et al., 2020). This effectively removes, or even reverses, any incentive to divide faster. This layer of control must be weakened by mutation or aging before oncogenes are selectively favored, and therapies that could restore or replace these controls could thus obviate the growth advantage of cancer cells. Chemotherapy that targets rapidly dividing cells provides a crude way to replace these controls, but at the cost of significant off-target side effects and evolution of resistance. The most explicit therapeutic use of this approach is the sucker's gambit (Merlo et al., 2006), which changes the selection pressures and incentive structures to select for phenotypes which are easier to treat. For example, increasing the concentration of glucose in a microenvironment changes the underlying incentive structure to favor cancer cells with high levels of GLUT1 receptors. Following this, administering glucose starvation or GLUT1 inhibitor treatment can force these cells to pay high and sometimes lethal costs for the production and maintenance of these receptors (Bukkuri and Brown, under review). Because cancer cells are short-sighted, successive administration of therapies that impose opposite evolutionary selection pressures on cancer cells can be effective. In ecology, conservation biologists seek to avoid "ecological traps," where species choose poor habitats when faced by novel species or habitat modifications (Schlaepfer et al., 2002), but such traps could tempt unwanted species and help with their control.

Treatment necessarily alters the benefit structure, and ideally can be used to sucker cancers into traps. One goal of modern therapies is to weaken the benefits of evolving resistance or evasion of therapy. We see analogies to education and public policy realms. Campbell's law states that the more a quantitative social marker is used for social decision making, the more it becomes subject to corruption that distorts the very social process it is intended to monitor (Campbell, 1979). For example, standardized testing can provide valuable information on student performace, but only when teaching is aimed at general competence. However, these quantitative measures soon became goals of the teaching process, subject to corruption that can actively degrade learning (Campbell, 1979). Schools and teachers face immense pressures to produce high test scores, particularly when tied to funding and bonuses (Nichols and Berliner, 2007), leading to "teaching to the test" (Popham, 2001) and elimination of subjects like social studies, music, foreign languages, and art from curricula (Byrd and Varga, 2018). These high stakes tests promote cheating, as discussed in the last subsection (Nichols and Berliner, 2007), and high-priced preparatory classes taken by students from more privileged backgrounds (Alon, 2009; Buchmann et al., 2010). Alternatives include more individualized assessments like portfolios (Kamenetz, 2015). We propose that the way that we assess cancers and choose treatments might be susceptible to Campbell's law. What if cancers start "growing to the test" and conceal their true size or state because it evades our treatment, almost the same way that cancer can evade immune responses?

## 7. DISCUSSION

If thought of narrowly as bribery, corruption provides a poor model for cancer. However, we argue that corruption is less about transfer of resources and more about breaking the communication system and disrupting the reliability of communication. In this sense, corruption is a violation of public trust (Wedel, 2012), the trust in signal integrity that any complex system relies on for coordination.

Evolutionary ecologists have identified five mechanisms that maintain the integrity of signaling systems: reduction of conflict of interest, costly signaling, saturating benefits, enforcement, and physical constraints. Each of these is paralleled in the body, and thus must be degraded by a surviving cancer. We propose examples of each of these mechanisms in ecology and in the body, and how they can be subverted. This approach provides an alternative view of the hallmarks of cancer.

We think this view proposes several directions for therapy, all of course building on prior work and ideas. First, rather than focusing on a single corrupted signal, we could use comprehensive approaches to recognize cancer through disrupted signaling (Krakauer and Pagel, 1996). Potentially dangerous lying, for example, can be recognized through the "too many details" that liars pile on to convince themselves and others (De Becker and Stechschulte, 1997). Cancer cells do not send off a carefully orchestrated set of consistent signals, but a welter of chemical noise that could be recognized through its very incoherence (Sur et al., 2019). From this recognition, we might be able to find ways to treat the resulting corruption of the communication network. The disappointing performance of VEGF inhibitors in effectively controlling cancer as monotherapy (Comunanza and Bussolino, 2017), for example, could reflect the challenge of placing such signal-disrupting therapies in the full context of the network.

By viewing cancer therapy as a public policy problem, we propose two main ways to combat corruption in our body: punishing corruption directly (the stick), changing the incentive structure so corrupt behavior is not favored (the carrot). As punishment, we focus on treatment as a form of taxation, showing how the very specificity and effectiveness of targeted therapies might make them subject to escape: the cancer equivalent of tax evasion. We advocate for careful consideration of the evolvability of the cancer when deciding whether to

administer traditional chemotherapy or targeted therapies. In the case of altering the incentive structure, we describe how oncogene-induced senescence and traditional chemotherapy can change the incentives for cancer cells to proliferate rapidly. We highlight how a sucker's gambit therapeutic strategy can combine these two approaches, tempting cancers with a carrot and then slamming them with a stick to promote maximal therapeutic efficacy.

Cancer treatment might benefit from other lessons from the challenges of fighting corruption in the economic and political spheres. Wedel (Wedel, 2012) describes the history of the anticorruption movement that emerged at the end of the cold war and found expression in Wolfensohn's speech to the World Bank in 1996. Of its four central assumptions, we find it remarkable that three (except for the focus on public rather than private sector corruption) have close parallels with cancer treatment, and propose that questioning these assumptions might provide new guidance for treatment.

1. Corruption happens to "the Other." Anticorruption efforts generally focus on distant nations with very different cultures from the centers of economic power. Not only do those of us fortunate enough to not have faced cancer tend to think "it can't happen to me," but one can imagine that the body itself sees an incipient cancer as happening elsewhere, rather than permeating the entire system. Treatment that revives the control mechanisms throughout the body, including sites of potential metastases, could stop the spread of cancers.
2. Corruption is about bribery to individuals, often at lower levels, rather than the system, and is illegal. Cancer treatment focuses on cells within the tumor itself, and on ways that cancers "break the rules" rather than on how they reshape the body at all levels. Like much corruption, such as campaign contributions, what cancers do is perfectly legal, and focusing on consequences and mechanisms of corruption could be more effective than a limited set of broken rules.
3. Corruption can be measured. This simple assumption reflects the famous saying by business management guru Peter Drucker, "If you can't measure it, you can't improve it." Indices, often derived from polls of business and political leaders with their own biases, make corruption easy to publicize in the media and compare across countries. As we have seen, indices are subject to Campbell's Law, effectively corrupting the evaluation mechanism itself. Treatments based on indices like specific biomarkers are subject to the same logic, favoring tumors that evade the evaluation mechanism itself (Staňková et al., 2019). More flexible cancer treatments can anticipate the evasion that can emerge when we use a specific marker to trigger treatment.

These approaches to corruption depend on the complexity of the signaling system. Ants in small colonies with individual foragers require and have fewer layers of control than those with extensive division of labor, just as smaller organisms like mice require and have fewer layers of cancer control than humans and elephants. We expect all aspects of control, ranging from costs, benefits, and enforcement to the modular structure of networks to also differ. It is possible that the challenges of extrapolating treatments from mice to humans are due as much to differences in these structural aspects as they are to differences in molecular details.

A different sort of modification of network functioning emerges with aging. The simultaneous degradation of signaling and of the full set of control systems likely causes the rapid increase of most cancers with age (DeGregori, 2018), and our treatments need to reflect this slow corruption of the integrity of the system. The danger of corruption in increasingly entrenched bureaucracies could reflect a similar process. As institutions develop into ever more complicated structures, corruption itself may become more unequal because only the privileged and well-connected can even figure out how to be corrupt.

In the long run, these general ideas need to be made concrete with mathematical models that build on the literature of corruption (Rose-Ackerman, 1975) and take a more comprehensive view of cancer that includes resources, signaling, and enforcement mechanisms in a framework that generates unexpected novelty (Adler and Gordon, 2019). When these models are linked to specific cancers, mechanisms, and treatments, they can be used to propose improved approaches to therapy that seek to restore balance to the whole patient.

## AUTHOR CONTRIBUTIONS

AB and FA contributed to the conceptualization, writing, simulations, and editing of this paper. Both authors contributed to the article and approved the submitted version.

## FUNDING

AB acknowledges support by the National Science Foundation Graduate Research Fellowship Program under Grant No. 1746051. FA acknowledges support from NIH-CSBC: U54: Combating subclonal evolution of resistant cancer phenotypes (U54 CA209978).

## ACKNOWLEDGMENTS

The authors thank Elizabeth Fedak and members of sLaM for useful insights and citations.

## REFERENCES

Adler, F. R., and Gordon, D. M. (2019). Cancer ecology and evolution: positive interactions and system vulnerability. *Curr. Opin. Syst. Biol.* 17, 1–7. doi: 10.1016/j.coisb.2019.09.001

Alberts, B., Johnson, A. D., Lewis, J., Morgan, D., Raff, M., Roberts, K., et al. (2014). *Molecular Biology of the Cell, 6th Edn*. New York, NY: W. W. Norton & Company.

Alon, S. (2009). The evolution of class inequality in higher education. *Am. Sociol. Rev.* 74, 731–755. doi: 10.1177/000312240907400503

Baeriswyl, V., and Christofori, G. (2009). The angiogenic switch in carcinogenesis. *Semin. Cancer Biol.* 19, 329–337. doi: 10.1016/j.semcancer.2009.05.003

Barnum, K. J., and O'Connell, M. J. (2014). Cell cycle regulation by checkpoints. *Methods Mol. Biol.* 1170, 29–40. doi: 10.1007/978-1-4939-0888-2_2

Bedikian, A. Y., Millward, M., Pehamberger, H., Conry, R., Gore, M., Trefzer, U., et al. (2006). BCL-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the oblimersen melanoma study group. *J. Clin. Oncol.* 24, 4738–4745. doi: 10.1200/JCO.2006.06.0483

Boisvert, A.-M. L., Dent, P., and Quraishi, O. B. (2014). Corruption in canada: definitions and enforcement. *Res. Brief* 48, 1–3.

Borcan, O., Lindahl, M., and Mitrut, A. (2017). Fighting corruption in education: what works and who benefits? *Am. Econ. J. Econ. Policy* 9, 180–209. doi: 10.1257/pol.20150074

Bowling, D. L., Garcia, M., Dunn, J. C., Ruprecht, R., Stewart, A., Frommolt, K. H., et al. (2017). Body size and vocalization in primates and carnivores. *Sci. Rep.* 7, 1–11. doi: 10.1038/srep41070

Buchmann, C., Condron, D. J., and Roscigno, V. J. (2010). Shadow education, american style: test preparation, the SAT and college enrollment. *Social Forces* 89, 435–461. doi: 10.1353/sof.2010.0105

Buschinger, A. (2009). Social parasitism among ants: a review (hymenoptera: Formicidae). *Myrmecol. News* 12, 219–235.

Butler, N. E., Magrath, R. D., and Peters, R. A. (2017). Lack of alarm calls in a gregarious bird: models and videos of predators prompt alarm responses but no alarm calls by zebra finches. *Behav. Ecol. Sociobiol.* 71, 1–13. doi: 10.1007/s00265-017-2343-z

Byrd, M., and Varga, B. A. (2018). The manifestation of Campbell's law: consequences of eliminating of social studies from the curriculum. *Soc. Stud.* 109, 27–33. doi: 10.1080/00377996.2017.1406322

Campbell, D. T. (1979). Assessing the impact of planned social change. *Eval. Prog. Plann.* 2, 67–90. doi: 10.1016/0149-7189(79)90048-X

Campisi, J., Gray, H. E., Pardee, A. B., Dean, M., and Sonenshein, G. E. (1984). Cell-cycle control of c-myc but not c-ras expression is lost following chemical transformation. *Cell* 36, 241–247. doi: 10.1016/0092-8674(84)90217-4

Carter, G. G., and Wilkinson, G. S. (2013). Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. *Proc. R. Soc. B Biol. Sci.* 280:20122573. doi: 10.1098/rspb.2012.2573

Catakovic, K., Klieser, E., Neureiter, D., and Geisberger, R. (2017). T cell exhaustion: from pathophysiological basics to tumor immunotherapy. *Cell Commun. Signal.* 15, 1–16. doi: 10.1186/s12964-016-0160-z

Cha, J. H., Chan, L. C., Li, C. W., Hsu, J. L., and Hung, M. C. (2019). Mechanisms controlling PD-L1 expression in cancer. *Mol. Cell* 76, 359–370. doi: 10.1016/j.molcel.2019.09.030

Cheney, D. L., and Seyfarth, R. M. (1988). Assessment of meaning and the detection of unreliable signals by vervet monkeys. *Anim. Behav.* 36, 477–486. doi: 10.1016/S0003-3472(88)80018-6

Cheng, H., Wang, Z., Fu, L., and Xu, T. (2019). Macrophage polarization in the development and progression of ovarian cancers: an overview. *Front. Oncol.* 9:421. doi: 10.3389/fonc.2019.00421

Clark, R., and Kimbrough, S. O. (2017). Social structure, opportunistic punishment and the evolution of honest signaling. *PLoS ONE* 12:e0188249. doi: 10.1371/journal.pone.0188249

Clutton-Brock, T. H., and Albon, S. D. (1979). The roaring of red deer and the evolution of honest advertisement. *Behaviour* 69, 145–170. doi: 10.1163/156853979X00449

Comunanza, V., and Bussolino, F. (2017). Therapy for cancer: strategy of combining anti-angiogenic and target therapies. *Front. Cell Dev. Biol.* 5:101. doi: 10.3389/fcell.2017.00101

Cotton, P. A., Kacelnik, A., and Wright, J. (1996). Chick begging as a signal: are nestlings honest? *Behav. Ecol.* 7, 178–182. doi: 10.1093/beheco/7.2.178

Dang, C. V. (2012). MYC on the path to cancer. *Cell* 149, 22–35. doi: 10.1016/j.cell.2012.03.003

De Becker, G., and Stechschulte, T. (1997). *The Gift of Fear*. Boston, MA: Little, Brown.

De Waal, F., and Waal, F. B. (2007). *Chimpanzee Politics: Power and Sex Among Apes*. London: JHU Press.

Dearborn, D. C. (1998). Begging behavior and food acquisition by brown-headed cowbird nestlings. *Behav. Ecol. Sociobiol.* 43, 259–270. doi: 10.1007/s002650050490

DeGregori, J. (2018). *Adaptive Oncogenesis: A New Understanding of How Cancer Evolves Inside US*. Cambridge, MA: Harvard University Press. doi: 10.4159/9780674985964

Detto, T., Jennions, M. D., and Backwell, P. R. (2010). When and why do territorial coalitions occur? Experimental evidence from a fiddler crab. *Am. Nat.* 175, E119–E125. doi: 10.1086/651588

Duijn, P. A., Kashirin, V., and Sloot, P. M. (2014). The relative ineffectiveness of criminal network disruption. *Sci. Rep.* 4, 1–15. doi: 10.1038/srep04238

Ellis, L. M., and Hicklin, D. J. (2009). Resistance to targeted therapies: refining anticancer therapy in the era of molecular oncology. *Clin. Cancer Res.* 15, 7471–7478. doi: 10.1158/1078-0432.CCR-09-1070

Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35, 495–516. doi: 10.1080/01926230701320337

Evans, C., and Evans, L. (2002). "*Sceptical hens: receivers contrain deceptive food calling by male fowl*," in *Animal Behavior Society Meeting* (Boise, ID).

Finver, S. N., Nishikura, K., Finger, L. R., Haluska, F. G., Finan, J., Nowell, P. C., et al. (1988). Sequence analysis of the MYC oncogene involved in the t(8;14)(q24;q11) chromosome translocation in a human leukemia T-cell line indicates that putative regulatory regions are not altered. *Proc. Natl. Acad. Sci. U.S.A.* 85, 3052–3056. doi: 10.1073/pnas.85.9.3052

Fisman, R., and Golden, M. (2017). How to fight corruption. *Science* 356, 803–804. doi: 10.1126/science.aan0815

Fuchs, Y., and Steller, H. (2011). Programmed cell death in animal development and disease. *Cell* 147, 742–758. doi: 10.1016/j.cell.2011.10.033

Garcia, R., Bowman, T. L., Niu, G., Yu, H., Minton, S., Muro-Cacho, C. A., et al. (2001). Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. *Oncogene* 20, 2499–2513. doi: 10.1038/sj.onc.1204349

Gatenby, R. A., Brown, J., and Vincent, T. (2009). Lessons from applied ecology: cancer control using an evolutionary double bind. *Cancer Res.* 69, 7499–7502. doi: 10.1158/0008-5472.CAN-09-1354

Geiselhardt, S. F., Peschke, K., and Nagel, P. (2007). A review of myrmecophily in ant nest beetles (coleoptera: Carabidae: Paussinae): linking early observations with recent findings. *Naturwissenschaften* 94, 871–894. doi: 10.1007/s00114-007-0271-x

Glaudas, X., Rice, S. E., Clark, R. W., and Alexander, G. J. (2020). The intensity of sexual selection, body size and reproductive success in a mating system with male-male combat: is bigger better? *Oikos* 129, 998–1011. doi: 10.1111/oik.07223

Gorgoulis, V. G., and Halazonetis, T. D. (2010). Oncogene-induced senescence: The bright and dark side of the response. *Curr. Opin. Cell Biol.* 22, 816–827. doi: 10.1016/j.ceb.2010.07.013

Grafen, A. (1990). Biological signals as handicaps. *J. Theoret. Biol.* 144, 517–546. doi: 10.1016/S0022-5193(05)80088-8

Griffiths, J. G., and Bonser, S. P. (2013). Is sex advantageous in adverse environments? *A test of the abandon-ship hypothesis. Am. Nat.* 182, 718–725. doi: 10.1086/673476

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Hare, J. F., and Atkins, B. A. (2001). The squirrel that cried wolf: Reliability detection by juvenile Richardson's ground squirrels (*Spermophilus richardsonii*). *Behav. Ecol. Sociobiol.* 51, 108–112. doi: 10.1007/s002650100414

Hasselquist, D. (1994). *Male attractiveness, mating tactics and realized fitness in the polygynous great reed warbler* (Ph.D. thesis). Lund University, Lund, Sweden.

Hauser, M. D. (1996). *The Evolution of Communication*. Cambridge: MIT Press.

Hoi-Leitner, M., Nechtelberger, H., and Hoi, H. (1995). Song rate as a signal for nest site quality in blackcaps (*Sylvia atricapilla*). *Behav. Ecol. Sociobiol.* 37, 399–405. doi: 10.1007/s002650050207

Hoshino, A., Costa-Silva, B., Shen, T. L., Rodrigues, G., Hashimoto, A., Tesic Mark, M., et al. (2015). Tumour exosome integrins determine organotropic metastasis. *Nature* 527, 329–335. doi: 10.1038/nature15756

Hough, K. P., Chanda, D., Duncan, S. R., Thannickal, V. J., and Deshane, J. S. (2017). Exosomes in immunoregulation of chronic lung diseases. *Allergy* 72, 534–544. doi: 10.1111/all.13086

Hua, Y., and Bergers, G. (2019). Tumors vs. *chronic wounds: an immune cell's perspective. Front. Immunol.* 10:2178. doi: 10.3389/fimmu.2019.02178

Hurwicz, L. (2007). *But who will guard the guardians*? Prize lecture. Nobel Prize Lecture.

Janeway, C. A., Travers, P., Walport, M., and Shlomchik, M. J. (2001). "Immunological memory," in *Immunobiology: The Immune System in Health and Disease, 5th Edn*, eds C. A. Janeway, Jr., and P. Travers (New York, NY: Garland Science).

Jiang, G., Wei, Z. P., Pei, D. S., Xin, Y., Liu, Y. Q., and Zheng, J. N. (2011). A novel approach to overcome temozolomide resistance in glioma and melanoma: inactivation of MGMT by gene therapy, volume 406. *Biochem. Biophys. Res. Commun.* 406, 311–314. doi: 10.1016/j.bbrc.2011.02.042

Jiang, Y., Li, Y., and Zhu, B. (2015). T-cell exhaustion in the tumor microenvironment. *Cell Death Dis.* 6:e1792. doi: 10.1038/cddis.2015.162

Johnstone, R. (1977). "The evolution of animal signals," in *Behavioral Ecology*, eds J. Krebs and N. Davies (Oxford: Blackwell), 155–178.

Joly, M. (2017). Corruption: the shortcut to disaster. *Sustain. Product. Consump.* 10, 133–156. doi: 10.1016/j.spc.2016.09.005

Kalluri, R., and LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science* 367:6478. doi: 10.1126/science.aau6977

Kamenetz, A. (2015). A review of: The Test: Why our schools are obsessed with standardized testing—but you don't have to be. *Wisdom in Educ.* 6:5. Available online at: https://scholarworks.lib.csusb.edu/wie/vol6/iss1/5

Kareva, I. (2015). Cancer ecology: niche construction, keystone species, ecological succession, and ergodic theory. *Biol. Theory* 10, 283–288. doi: 10.1007/s13752-015-0226-y

Kastan, M. B., and Bartek, J. (2004). Cell-cycle checkpoints and cancer. *Nature* 432, 316–323. doi: 10.1038/nature03097

Kilner, R. M., Noble, D. G., and Davies, N. B. (1999). Signals of need in parent-offspring communication and their exploitation by the common cuckoo. *Nature* 397, 667–672. doi: 10.1038/17746

Kleiven, O. T., Larsson, P., Hobæk, A., and Hobaek, A. (1992). Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos* 65:197. doi: 10.2307/3545010

Kolokoltsov, V. N., and Malafeyev, O. A. (2017). Mean-field-game model of corruption. *Dyn. Games Appl.* 7, 34–47. doi: 10.1007/s13235-015-0175-x

Korolev, K. S., Xavier, J. B., and Gore, J. (2014). Turning ecology and evolution against cancer. *Nat. Rev. Cancer* 14, 371–380. doi: 10.1038/nrc3712

Krakauer, D. C., and Pagel, M. (1996). Selection by somatic signals: the advertisement of phenotypic state through costly intercellular signals. *Philos. Trans. Biol. Sci.* 351, 647–658. doi: 10.1098/rstb.1996.0062

Kumar, H., Kawai, T., and Akira, S. (2011). Pathogen recognition by the innate immune system. *Int. Rev. Immunol.* 30, 16–34. doi: 10.3109/08830185.2010.529976

Lage, H., Christmann, M., Kern, M. A., Dietel, M., Pick, M., Kaina, B., et al. (1999). Expression of DNA repair proteins hMSH2, hMSH6, hMLH1, O6-methylguanine-DNA methyltransferase and N-methylpurine-DNA glycosylase in melanoma cells with acquired drug resistance. *Int. J. Cancer* 80, 744–750. doi: 10.1002/(SICI)1097-0215(19990301)80:5<744::AID-IJC19>3.0.CO;2-5

Lederberg, J. (1999). JBS Haldane (1949) on infectious disease and evolution. *Genetics* 153, 1–3. doi: 10.1093/genetics/153.1.1

Lenoir, A., d'Ettorre, P., Errard, C., and Hefetz, A. (2001). Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol.* 46, 573–599. doi: 10.1146/annurev.ento.46.1.573

Lev, D. C., Ruiz, M., Mills, L., McGary, E. C., Price, J. E., and Bar-Eli, M. (2003). Dacarbazine causes transcriptional up-regulation of interleukin 8 and vascular endothelial growth factor in melanoma cells: a possible escape mechanism from chemotherapy. *Mol. Cancer Therapeut.* 2, 753–763.

Levin, M., and Tsirik, M. (1998). Corruption as an object of mathematical modeling. *Matekon* 34, 16–46.

Levine, E. M., Becker, Y., Boone, C. W., and Eagle, H. (1965). Contact inhibition, macromolecular synthesis, and polyribosomes in cultured human diploid fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.* 53, 350–356. doi: 10.1073/pnas.53.2.350

Li, X.-B., Zhang, Z.-R., Schluesener, H. J., and Xu, S.-Q. (2006). Role of exosomes in immune regulation. *J. Cell. Mol. Med.* 10, 364–375. doi: 10.1111/j.1582-4934.2006.tb00405.x

Liberti, M. V., and Locasale, J. W. (2016). The Warburg effect: how does it benefit cancer cells? *Trends Biochem. Sci.* 41, 211–218. doi: 10.1016/j.tibs.2015.12.001

Liu, X., l., Ding, J., and Meng, L., h. (2018). Oncogene-induced senescence: a double edged sword in cancer. *Acta Pharmacol. Sin.* 39, 1553–1558. doi: 10.1038/aps.2017.198

Luna-Pla, I., and Nicolás-Carlock, J. R. (2020). Corruption and complexity: a scientific framework for the analysis of corruption networks. *Appl. Netw. Sci.* 5, 1–18. doi: 10.1007/s41109-020-00258-2

Madan, E., Gogna, R., and Moreno, E. (2018). Cell competition in development: information from flies and vertebrates. *Curr. Opin. Cell Biol.* 55, 150–157. doi: 10.1016/j.ceb.2018.08.002

Madan, E., Pelham, C. J., Nagane, M., Parker, T. M., Canas-Marques, r., Fazio, K., et al. (2019). Flower isoforms promote competitive growth in cancer. *Nature.* 572, 260–264. doi: 10.1038/s41586-019-1429-3

Manica, L. T., Maia, R., Dias, A., Podos, J., and Macedo, R. H. (2014). Vocal output predicts territory quality in a neotropical songbird. *Behav. Process.* 109, 21–26. doi: 10.1016/j.beproc.2014.07.004

Martincorena, I., Roshan, A., Gerstung, M., Ellis, P., Van Loo, P., McLaren, S., et al. (2015). High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 348, 880–886. doi: 10.1126/science.aaa6806

Maverakis, E., Kim, K., Shimoda, M., Gershwin, M. E., Patel, F., Wilken, R., et al. (2015). Glycans in the immune system and The Altered Glycan theory of autoimmunity: a critical review. *J. Autoimmun.* 57, 1–13. doi: 10.1016/j.jaut.2014.12.002

Maynard-Smith, J. (1991). Honest signalling: the Philip Sidney game. *Anim. Behav.* 42, 1034–1035. doi: 10.1016/S0003-3472(05)80161-7

Maynard-Smith, J., and Harper, D. (2003). *Animal Signals*. New York, NY: Oxford University Press.

Maziveyi, M., Dong, S., Baranwal, S., Mehrnezhad, A., Rathinam, R., Huckaba, T. M., et al. (2019). Exosomes from nischarin-expressing cells reduce breast cancer cell motility and tumor growth. *Cancer Res.* 79, 2152–2166. doi: 10.1158/0008-5472.CAN-18-0842

Merino, M. M., Rhiner, C., Lopez-Gay, J. M., Buechel, D., Hauert, B., and Moreno, E. (2015). Elimination of unfit cells maintains tissue health and prolongs lifespan. *Cell* 160, 461–476. doi: 10.1016/j.cell.2014.12.017

Merino, M. M., Rhiner, C., Portela, M., and Moreno, E. (2013). "Fitness fingerprints" *mediate physiological culling of unwanted neurons in Drosophila. Curr. Biol.* 23, 1300–1309. doi: 10.1016/j.cub.2013.05.053

Merlo, L. M., Pepper, J. W., Reid, B. J., and Maley, C. C. (2006). Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer* 6, 924–935. doi: 10.1038/nrc2013

Milojkovic, D., and Apperley, J. F. (2009). Mechanisms of resistance to imatinib and second-generation tyrosine inhibitors in chronic myeloid leukemia. *Clin. Cancer Res.* 15, 7519–7527. doi: 10.1158/1078-0432.CCR-09-1068

Mitchell, S. E., Read, A. F., and Little, T. J. (2004). The effect of a pathogen epidemic on the genetic structure and reproductive strategy of the crustacean *Daphnia magna. Ecol. Lett.* 7, 848–858. doi: 10.1111/j.1461-0248.2004.00639.x

Møller, A. P. (2010). False alarm calls as a means of resource usurpation in the great tit parus major. *Ethology* 79, 25–30. doi: 10.1111/j.1439-0310.1988.tb00697.x

Monnin, T., and Ratnieks, F. L. (2001). Policing in queenless ponerine ants. *Behav. Ecol. Sociobiol.* 50, 97–108. doi: 10.1007/s002650100351

Moreno, E., Fernandez-Marrero, Y., Meyer, P., and Rhiner, C. (2015). Brain regeneration in Drosophila involves comparison of neuronal fitness. *Curr. Biol.* 25, 955–963. doi: 10.1016/j.cub.2015.02.014

Morton, E. S. (1977). On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. *Am. Nat.* 111, 855–869. doi: 10.1086/283219

Nagel, D., Vincendeau, M., Eitelhuber, A. C., and Krappmann, D. (2014). Mechanisms and consequences of constitutive NF-$\kappa$B activation in B-cell lymphoid malignancies. *Oncogene* 33, 5655–5665. doi: 10.1038/onc.2013.565

Nekovee, M., and Pinto, J. (2019). Modeling the impact of organization structure and whistle-blowers on intra-organizational corruption contagion. *Phys. A Stat. Mech. Appl.* 522, 339–349. doi: 10.1016/j.physa.2019.01.140

Nichols, S. L., and Berliner, D. C. (2007). "The pressure to cheat in a high-stakes testing environment," in *Psychology of Academic Cheating*, eds E. M. Anderman and T. B. Murdock (Amsterdam: Elsevier Inc.), 289–311. doi: 10.1016/B978-012372541-7/50016-4

Ombrato, L., Nolan, E., Kurelac, I., Mavousian, A., Louise Bridgeman, V., Heinze, I., et al. (2019). Metastatic-niche labelling reveals parenchymal cells with stem features. *Nature* 572, 603–608. doi: 10.1038/s41586-019-1487-6

Otte, D. (1974). Effects and functions in the evolution of signaling systems. *Annu. Rev. Ecol. Syst.* 5, 385–417. doi: 10.1146/annurev.es.05.110174.002125

Park, J. E., Tan, H. S., Datta, A., Lai, R. C., Zhang, H., Meng, W., et al. (2010). Hypoxic tumor cell modulates its microenvironment to enhance angiogenic and metastatic potential by secretion of proteins and exosomes. *Mol. Cell. Proteom.* 9, 1085–1099. doi: 10.1074/mcp.M900381-MCP200

Pavel, M., Renna, M., Park, S. J., Menzies, F. M., Ricketts, T., Füllgrabe, J., et al. (2018). Contact inhibition controls cell survival and

proliferation via YAP/TAZ-autophagy axis. *Nat. Commun.* 9, 1–18. doi: 10.1038/s41467-018-05388-x

Pienta, K. J., Hammarlund, E. U., Brown, J. S., Amend, S. R., and Axelrod, R. M. (2021). Cancer recurrence and lethality are enabled by enhanced survival and reversible cell cycle arrest of polyaneuploid cells. *Proc. Natl. Acad. Sci. U.S.A.* 118:e2020838118. doi: 10.1073/pnas.2020838118

Pienta, K. J., McGregor, N., Axelrod, R., and Axelrod, D. E. (2008). Ecological therapy for cancer: defining tumors using an ecosystem paradigm suggests new opportunities for novel cancer treatments. *Transl. Oncol.* 1, 158–164. doi: 10.1593/tlo.08178

Pohlmann, P. R., Mayer, I. A., and Mernaugh, R. (2009). Resistance to trastuzumab in breast cancer. *Clin. Cancer Res.* 15, 7479–7491. doi: 10.1158/1078-0432.CCR-09-0636

Popham, W. J. (2001). Teaching to the test. *Educ. Leadership* 58, 16–20.

Ribatti, D., Nico, B., Crivellato, E., Roccaro, A. M., and Vacca, A. (2007). The history of the angiogenic switch concept. *Leukemia* 21, 44–52. doi: 10.1038/sj.leu.2404402

Rose-Ackerman, S. (1975). The economics of corruption. *J. Public Econ.* 4, 187–203. doi: 10.1016/0047-2727(75)90017-1

Ryan, M. J., Fox, J. H., Wilczynski, W., and Rand, A. S. (1990). Sexual selection, sensory systems and sensory exploitation. *Oxford Surv. Evol. Biol.* 7, 157–195.

Rytkonen, S. O., Orell, M. I., Koivula, K. T., and Welling, P. P. (1997). Song rate correlates with paternal care and survival in willow tits: advertisement of male quality? *Behaviour*. 134, 891–904. doi: 10.1163/156853997X00214

Sacchi, R. (2002). Features of begging calls reveal general condition and need of food of barn swallow (*Hirundo rustica*) nestlings. *Behav. Ecol.* 13, 268–273. doi: 10.1093/beheco/13.2.268

Schietinger, A., and Greenberg, P. D. (2014). Tolerance and exhaustion: defining mechanisms of T cell dysfunction. *Trends Immunol.* 35, 51–60. doi: 10.1016/j.it.2013.10.001

Schlaepfer, M. A., Runge, M. C., and Sherman, P. W. (2002). Ecological and evolutionary traps. *Trends Ecol. Evol.* 17, 474–480. doi: 10.1016/S0169-5347(02)02580-6

Schmid, M. C., and Varner, J. A. (2012). Myeloid cells in tumor inflammation. *Vasc. Cell* 4:14. doi: 10.1186/2045-824X-4-14

Schmidt-Nielsen, K. (1984). *Scaling, Why Is Animal Size So Important?* New York, NY: Cambridge University Press. doi: 10.1017/CBO9781139167826

Searcy, W. A., and Nowicki, S. (2005). *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton, NJ: Princeton University Press.

Seliger, B. (2005). Strategies of tumor immune evasion. *Biodrugs* 19, 347–354. doi: 10.2165/00063030-200519060-00002

Shine, R. (1990). Function and evolution of the frill of the frillneck lizard, *Chlamydosaurus kingii* (sauria: Agamidae). *Biol. J. Linnean Soc.* 40, 11–20. doi: 10.1111/j.1095-8312.1990.tb00531.x

Silk, J. B., Kaldor, E., and Boyd, R. (2000). Cheap talk when interests conflict. *Anim. Behav.* 59, 423–432. doi: 10.1006/anbe.1999.1312

Slamon, D. J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., et al. (2001). Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 344, 783–792. doi: 10.1056/NEJM200103153441101

Staňková, K., Brown, J. S., Dalton, W. S., and Gatenby, R. A. (2019). Optimizing cancer treatment using game theory: a review. *JAMA Oncol.* 5, 96–103. doi: 10.1001/jamaoncol.2018.3395

Stegelmeier, A. A., van Vloten, J. P., Mould, R. C., Klafuric, E. M., Minott, J. A., Wootton, S. K., et al. (2019). Myeloid cells during viral infections and inflammation. *Viruses* 11:1. doi: 10.3390/v11020168

Strauss, L., Guarneri, V., Gennari, A., and Sica, A. (2020). Implications of metabolism-driven myeloid dysfunctions in cancer therapy. *Cell. Mol. Immunol.* 18, 1–13. doi: 10.1038/s41423-020-00556-w

Sur, D. G., Colceriu, M., Sur, G., Aldea, C., Silaghi, C., Samasca, G., et al. (2019). MiRNAs roles in the diagnosis, prognosis and treatment of colorectal cancer. *Expert Rev. Proteom.* 16, 851–856. doi: 10.1080/14789450.2019.1659732

Swann, J. B., and Smyth, M. J. (2007). Immune surveillance of tumors. *J. Clin. Investig.* 117, 1137–1146. doi: 10.1172/JCI31405

Tansey, W. P. (2014). Mammalian MYC proteins and cancer. *N. J. Sci.* 2014:757534. doi: 10.1155/2014/757534

Tawbi, H. A., and Buch, S. C. (2010). Chemotherapy resistance abrogation in metastatic melanoma. *Clin. Adv. Hematol. Oncol.* 8, 259–266.

Temeles, E. J. (1994). The role of neighbours in territorial systems: when are they "dear enemies?" *Anim. Behav.* 47, 339–350. doi: 10.1006/anbe.1994.1047

Tibbetts, E. A., and Dale, J. (2004). A socially enforced signal of quality in a paper wasp. *Nature* 432, 218–222. doi: 10.1038/nature02949

Tibbetts, E. A., and Izzo, A. (2010). Social punishment of dishonest signalers caused by mismatch between signal and behavior. *Curr. Biol.* 20, 1637–1640. doi: 10.1016/j.cub.2010.07.042

Tsukumo, S. I., and Yasutomo, K. (2018). Regulation of CD8+ T cells and antitumor immunity by Notch signaling. *Front. Immunol.* 9:101. doi: 10.3389/fimmu.2018.00101

Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009). Understanding the warburg effect: The metabolic requirements of cell proliferation. *Science* 324, 1029–1033. doi: 10.1126/science.1160809

Vehrencamp, S. (2000). *Handicap, Index, and Conventional Signal Elements of Bird Song*. Trondheim: Tapir Academic Press.

Vehrencamp, S. L. (2009). "Animal communication: honesty and deception," in *Encyclopedia of Neuroscience*, ed L. R. Squire (Berlin: Academic Press), 397–406. doi: 10.1016/B978-008045046-9.01835-0

Vinay, D. S., Ryan, E. P., Pawelec, G., Talib, W. H., Stagg, J., Elkord, E., et al. (2015). Immune evasion in cancer: mechanistic basis and therapeutic strategies. *Semin. Cancer Biol.* 35, S185–S198. doi: 10.1016/j.semcancer.2015.03.004

Watson, K. A., and Ritchison, G. (2018). Effect of variation in nestling hunger levels on the begging behaviour of nestlings and the provisioning behaviour of adult American kestrels. *Avian Biol. Res.* 11, 35–43. doi: 10.3184/175815617X15127411523971

Wedel, J. R. (2012). Rethinking corruption in an age of ambiguity. *Annu. Rev. Law Soc. Sci.* 8, 453–498. doi: 10.1146/annurev.lawsocsci.093008.131558

Wei, S.-J. (1999). "Corruption in economic development: beneficial grease, minor annoyance, or major obstacle?" in *World Bank Policy Research Working Paper*. doi: 10.1596/1813-9450-2048

Wherry, E. J. (2011). T cell exhaustion. *Nature Immunol.* 12, 492–499. doi: 10.1038/ni.2035

Wilts, B. D., Michielsen, K., De Raedt, H., and Stavenga, D. G. (2014). Sparkling feather reflections of a bird-of-paradise explained by finite-difference time-domain modeling. *Proc. Natl. Acad. Sci. U.S.A.* 111, 4363–4368. doi: 10.1073/pnas.1323611111

Wolfensohn, J. D. (2005). *Voice for the World's Poor: Selected Speeches and Writings of World Bank President James D. Wolfensohn, 1995-2005*, Vol. 889. World Bank Publications. doi: 10.1596/0-8213-6156-2

Yom-Tov, Y. (1980). Intraspecific nest parasitism in birds. *Biol. Rev.* 55, 93–108. doi: 10.1111/j.1469-185X.1980.tb00689.x

Zhang, Z., Liu, S., Zhang, B., Qiao, L., Zhang, Y., and Zhang, Y. (2020). T cell dysfunction and exhaustion in cancer. *Front. Cell Dev. Biol.* 8:17. doi: 10.3389/fcell.2020.00017

Zhu, H., Blake, S., Kusuma, F. K., Pearson, R. B., Kang, J., and Chan, K. T. (2020). Oncogene-induced senescence: from biology to therapy. *Mech. Ageing Dev.* 187:111229. doi: 10.1016/j.mad.2020.111229

Zindl, C. L., and Chaplin, D. D. (2010). Tumor immune evasion. *Science* 328, 697–698. doi: 10.1126/science.1190310

**Disclaimer:** Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

PERSPECTIVE
published: 15 July 2021
doi: 10.3389/fevo.2021.676802

# The Evolutionary Ecology of Dormancy in Nature and in Cancer

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Dormancy is an inactive period of an organism's life cycle that permits it to survive through phases of unfavorable conditions in highly variable environments. Dormancy is not binary. There is a continuum of dormancy phenotypes that represent some degree of reduced metabolic activity (hypometabolism), reduced feeding, and reduced reproduction or proliferation. Similarly, normal cells and cancer cells exhibit a range of states from quiescence to long-term dormancy that permit survival in adverse environmental conditions. In contrast to organismal dormancy, which entails a reduction in metabolism, dormancy in cells (both normal and cancer) is primarily characterized by lack of cell division. "Cancer dormancy" also describes a state characterized by growth stagnation, which could arise from cells that are not necessarily hypometabolic or non-proliferative. This inconsistent terminology leads to confusion and imprecision that impedes progress in interdisciplinary research between ecologists and cancer biologists. In this paper, we draw parallels and contrasts between dormancy in cancer and other ecosystems in nature, and discuss the potential for studies in cancer to provide novel insights into the evolutionary ecology of dormancy.

**Keywords: dormancy, cancer, hypometabolism, quiescence, adaptation**

OPEN ACCESS

***Edited by:***
*Sasha Raoul Xola Dall, University of Exeter, United Kingdom*

***Reviewed by:***
*Thomas Haaland, University of Zurich, Switzerland*
*E. Yagmur Erten, University of Zurich, Switzerland*
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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *06 March 2021*
***Accepted:*** *16 June 2021*
***Published:*** *15 July 2021*

***Citation:***
*Miller AK, Brown JS, Enderling H, Basanta D and Whelan CJ (2021) The Evolutionary Ecology of Dormancy in Nature and in Cancer. Front. Ecol. Evol. 9:676802. doi: 10.3389/fevo.2021.676802*

## INTRODUCTION

"*You keep using that word. I do not think it means what you think it means.*" From the character Inigo Montoya, *Princess Bride*.

Most living organisms, from microbes to blue whales, experience temporal environmental fluctuations. These fluctuations induce periods of stress due to extreme temperatures, lack of resources, or disease. Dormancy, an evolutionary adaptation, enables organisms to survive through stressful conditions, in part by decreasing metabolic activity to conserve energy. However, there is a broad range of dormant states across taxa, which vary based on how long an organism remains in dormancy, whether dormancy is induced prior to or in response to stress, the magnitude of the response, etc. Despite attempts to devise a universal framework for animal dormancy (Wilsterman et al., 2020), precise definitions remain challenging. Dormancy is further complicated by diverging conceptualizations in other fields.

Cancer dormancy often refers to a period of time, from months, years, or even decades, between treatment of a primary tumor and metastatic relapse. This could be due to isolated non-proliferative cells that disseminated from the primary tumor (**cancer cell dormancy**) or small non-expanding populations of cancer cells (**tumor mass dormancy**). Although both of these categories could result in clinically undetectable cancer, they describe different biological mechanisms. This creates confusion within the field about what is meant by "cancer dormancy," and between fields about how cancer dormancy relates to organismal dormancy in other species. Furthermore, additional terminology has been used to describe cancer cells that survive treatment or other cellular stresses such as drug-tolerant persisters, hypoxia-resistant cells, and polyaneuploid cancer cells (PACCs). These descriptors and cell states add to the confusion owing to their overlapping characteristics with dormant cancer cells, such as cellular quiescence. Furthermore, this babel of terms and cell states can stifle the exchange of ideas between cancer biologists and other evolutionary ecologists.

In this paper, we take a critical look at the concept of tumor cell dormancy, in its many guises. Although we are not the first to point out the confusion associated with tumor cell dormancy (Vallette et al., 2019; Phan and Croucher, 2020), we aim to help clarify terminology by comparing it to key characteristics of dormancy in nature. This is not to imply that cancer is not a part of nature. It is. Rather we will use "in nature" as shorthand to describe all natural systems other than cancer. In what follows, we first examine how the term dormancy and related concepts are used in organismal biology. We then examine the history of the terms in cancer biology, with a focus on how those uses compare to what the terms mean in the context of whole organism biology and ecology. Finally, we discuss the mutual benefit of studying dormancy as it applies to ecology and cancer biology, and how experiments in cancer may help provide novel insights into mechanisms that drive dormancy from cells to organisms. Overall, we hope this paper provides a starting point for ecologists to help understand the terminology used in cancer biology and facilitate cross-disciplinary work on dormancy, while simultaneously convincing cancer biologists of the benefits of conceptualizing cancer dormancy using insights from ecology.

## DORMANCY IN ORGANISMAL BIOLOGY

Dormancy is often used as an "umbrella" term indicating a spectrum of inactive states characterized by reduced metabolism, or hypometabolism, as adaptations to survive periods of reduced resource availability or other adverse environmental conditions. Dormancy also refers to specific states of hypometabolism in both animals and plants (**Table 1**). The terms "**dormancy**" and "**torpor**" are often used interchangeably in the organismal literature. Dormancy encompasses many different hypometabolic states that have evolved across widely divergent taxa. These hypometabolic states exist along a continuum of metabolic expenditure (**Figure 1**). From the extreme state of essentially zero metabolic expenditure exhibited by bacterial endospores and cysts, dormant states include seed dormancy, estivation,

**TABLE 1 |** Definitions of terms used in reference to states of reduced activity and/or metabolism in cancer, microbiology, animals, and plants.

| Term | Definition |
|---|---|
| Aestivation (estivation) | In **insects**, a state of reduced metabolic activity and reduced physical activity in response to arid conditions and high temperatures. In **lungfish**, a period of reduced feeding, respiration, and movement in response to prolonged drought and heat. |
| Brumation | Winter dormancy of **ectotherms** like **reptiles and amphibians** that is induced by low temperature. |
| Cyst | A thick-walled, dormant structure produced by some **bacteria and protozoa** capable of enduring challenging environmental conditions, such as desiccation or high temperatures. |
| Diapause | In **insects**, a state of delayed or suspended development or growth, accompanied by reduced metabolic activity, that is part of a developmental program and not triggered directly by adverse environmental conditions. |
| Dormancy | A state of hypometabolism used by **animals and plants** to survive through adverse environmental conditions. Often used interchangeably with torpor. In **animals**, dormancy can be of short duration with only slight decreases in body temperature and metabolic activity (shallow dormancy), or of long duration with great decrease in body temperature and metabolic activity (deep dormancy). In **plants**, dormancy is a state in which **seeds** or other tissue reduce metabolic activity and cease growth during hostile environmental conditions, such as winter or drought. In **cancer**, may refer to tumor mass dormancy or tumor cell dormancy. Tumor mass dormancy occurs when cancer cell proliferation is balanced by cell death, resulting in a non-expanding population of cancer cells. This could happen due to poor vascularization (angiogenic dormancy) or control by the immune system (immune-mediated dormancy). Tumor cell dormancy is a state in which cells are quiescent and are often assumed to have low metabolism. |
| Encystment | Production of a resistant stage known as a cyst in some **bacteria, protozoa, plankton, and some invertebrates**, such as **flatworms**. |
| Endospore | A resistant, dormant and non-reproductive structure produced by some **bacteria** in the Firmicute family that ensure survival through hostile environmental conditions. |
| Hibernation | A state of greatly reduced activity, body temperature, metabolic rate, usually entered seasonally. |
| Hypo-metabolism | A state of reduced metabolic activity that includes a coordinated suppression of most cell functions; may vary in duration. |
| Quiescence | In **insects**, a temporary reduction in metabolic activity and a slowing or halt to development in response to adverse environmental conditions. In **plants**, a repression of division in undifferentiated cells in a plant's meristem (tissue from which new cells are formed). In **cells**, a reversible, non-proliferative state during which cells are in the $G_0$ phase of the cell cycle. |
| Resting egg | An **invertebrate** egg that undergoes a period of dormancy during which it is highly resistant to environmental conditions. |
| Torpor | A state of lowered body temperature and metabolic activity generally in response to challenging environmental conditions; may be of short duration (hours), as in nightly torpor of hummingbirds, or of long duration (days to weeks), as in hibernation of many ground squirrel species. Often used interchangeably with dormancy. |

*Where the word has different meanings contingent upon the organism or biological application (bold), we include separate definitions for each. "Sharp lines and precise definitions" for many of these terms do not exist, and many of these states may be considered as part of a "coherent physiological phenomenon" (Schmidt-Nielsen, 1997, p. 279).*

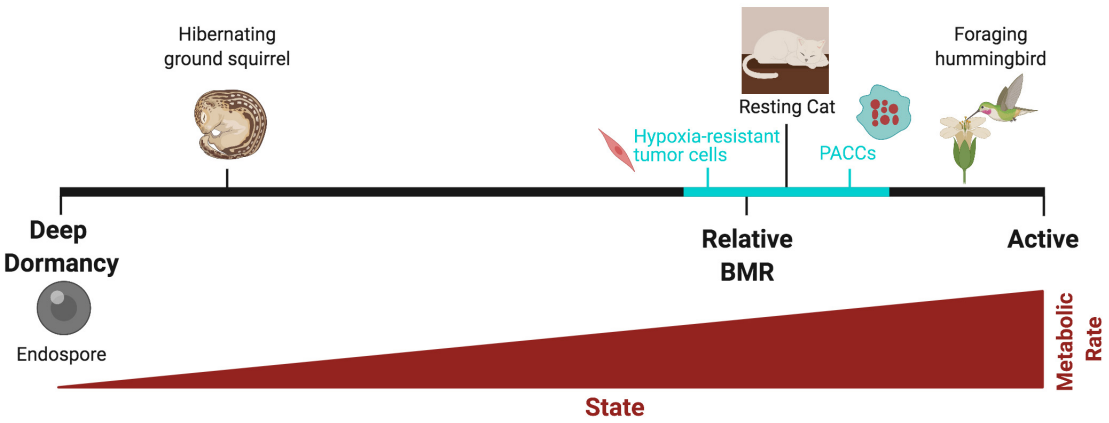


**FIGURE 1 |** Continuum of metabolic states in organisms and cancer cells, from lowest ("deep dormancy") to highest ("active"). The states below basal metabolic rate (BMR) represent hypometabolic states. The metabolic continuum of cancer cells (teal) likely falls within a narrower range of metabolic states in organisms (black). Created with BioRender.com.

diapause, quiescence, and hibernation. These diverse states are difficult to place into well-defined categories with sharp boundaries, and terminology can be confusing (Schmidt-Nielsen, 1997; Lee, 2009). Some of the characteristics that differentiate these states include (1) whether an organism enters dormancy prior to (obligatory) or in direct response to (facultative) an environmental stress, (2) the duration of the response (3) the reduction in metabolic activity, and (4) how resistant the state is to predation or stress. Dormancy may be of short duration (hours), as in the shallow or daily torpor of hummingbirds, or of long duration (days to weeks to months to years), as in the deep torpor associated with hibernation in mammals or seed dormancy in plants (Melvin and Andrews, 2009). While the manifestation of these states differs considerably among taxa, dormancy shares various core elements across numerous taxa (Melvin and Andrews, 2009; Villanueva-Cañas et al., 2014). Shared elements include an integrated down-regulation of cell functions, including cross-membrane transport, intermediary metabolism (biochemical reactions that provide the cell with metabolic intermediates), gene expression and protein synthesis, and utilization of stored energy reserves. In the following, we describe a subset of dormant states in organisms in order to give a broad overview of the terminology, and include a more complete set in **Table 1**. Although dormancy clearly is an adaptation to minimize energy expenditure during adverse environmental conditions, it comes with some costs, including the cost of arousal through endogenous heat production and vulnerability to predators (Withers and Cooper, 2010).

Most plant species produce seeds that exhibit a period of complete metabolic **dormancy** following dispersal from the mother plant (Bewley, 1997; Baskin and Baskin, 2004). In temperate regions and deserts, many plants themselves enter a dormant stage (Rohde and Bhalerao, 2007), in which photosynthetic and other metabolic activity cease or are reduced to very low levels. In winter dormancy, woody plants like trees and shrubs typically drop their leaves, and many herbaceous plants survive the winter below ground as roots while letting their aboveground biomass whither.

Many microorganisms, including bacteria and protists, differentiate into a metabolically inactive and highly resistant state when faced with starvation or inhospitable environmental conditions (Sadoff, 1975). The most resistant state, produced by some bacteria, is known as an **endospore**. The endospore may exist for centuries, during which time they "exhibit complete metabolic dormancy and extreme resistance to multiple environmental insults" (Mury and Popham, 2014). A similar resistant state, known as a **cyst**, **resting egg**, or **resting stage**, is also common in a variety of protists (Corliss, 2001; Ross and Hallock, 2016), and phyto- (Ribeiro et al., 2011; Ellegaard and Ribeiro, 2018) and zooplankton (Gilbert, 1974; Ricci, 2001).

Among insects, **estivation** is a dormant state that manifests as **quiescence,** a short period of moderately depressed metabolic rate triggered by unfavorable environmental conditions (Masaki, 2009); or **diapause**, a prolonged period of suppressed metabolism and arrested development (Denlinger, 2009; Lee, 2009). Diapause may be facultative or obligatory, and generally is expressed in a particular life stage, which varies among taxa. Depending on the taxa, the egg, larval, or adult life history stage may undergo diapause.

Perhaps the type of dormancy that is most familiar among people generally is **hibernation (deep torpor)**— classically exhibited by ground squirrels (e.g., Punxsutawney Phil of the movie *Groundhog Day*) and carnivores like bears. Hibernation in this sense appears inextricably tied to endothermy, as hibernation involves a shallow to a deep decline in body temperature (Lyman et al., 1982). In its most extreme manifestation thus documented, the arctic ground squirrel (*Urocitellus parryii*) drops its core body temperature from 37 to −1.9°C during its nine month hibernation (Barnes, 1989). Because many species inhabiting subtropical and tropical

regions exhibit hypometabolism generally associated with winter dormancy, many investigators now believe that hibernation is a flexible phenotypic response to scarce resources and energy conservation instead of a direct response to cold temperatures (Martin and Yoder, 2014).

## QUIESCENCE AND DORMANCY IN NORMAL CELLS

As with whole organisms, quiescence and dormancy are terms commonly used to describe a growth arrested state in mammalian cells including hematopoietic stem cells, lymphocytes, and fibroblasts. **Quiescence** is a reversible, non-proliferative state in response to nutrient deprivation (e.g., glucose, insulin, amino acids), mitogen or growth factor deprivation, loss of adhesion, or contact inhibition (Valcourt et al., 2012; Yao, 2014). Quiescence is essential for tissue homeostasis and regulation of the immune and wound healing response (Valcourt et al., 2012; Fiore et al., 2018). Gene expression, metabolism, and cell cycle re-entrance dynamics vary widely among quiescent clonal cell populations depending on the signal that initiated quiescence. As with dormancy in organisms, quiescence describes a collection of diverse states (Yao, 2014). However, cellular quiescence may not include hypometabolism, or a shutting down of metabolic functions outside of proliferation.

Hematopoietic stem cells (HSCs) regulate hematopoiesis, the production of billions of blood cells each day. *In vivo* mouse studies suggest that there are two populations of HSCs that control homeostasis and are maintained in adjacent "niches". Short-term ("active") HSCs are capable of self-renewal and divide frequently to replenish blood cells daily. Long-term HSCs may divide only five or so times per lifetime, or they can be activated to proliferate in response to injury. These long-term HSCs have been termed "**dormant**" (Wilson et al., 2008; Li and Clevers, 2010). Dormant HSCs exhibit a decreased metabolism as a result of reduced ribosomal biogenesis and DNA replication and are highly dependent on autophagy for survival (Wilson et al., 2008; Valcourt et al., 2012). Maintaining dormant HSCs is evolutionarily advantageous because it decreases the risk for oncogenic mutations and helps prevent stem cell depletion (Wilson et al., 2008).

Further down the hematopoietic lineage, lymphocytes, components of the adaptive immune response, are maintained in a **quiescent state** until activation by antigen presentation (Bryder et al., 2006). Quiescent lymphocytes are small in size and have few membrane glucose transporters, especially in the absence of growth factors (Valcourt et al., 2012). Quiescent lymphocytes depend on autophagy to obtain carbon sources for ATP production, which is synthesized by oxidative phosphorylation (Valcourt et al., 2012). Upon activation, glucose transporters increase and lymphocytes produce ATP by glycolysis (Valcourt et al., 2012). Lymphocyte quiescence prevents cell exhaustion and autoimmune disease.

Human dermal fibroblasts are maintained in a **quiescent state** that is characterized by their secretion of extracellular matrix. Wounding induces fibroblast activation and proliferation to coordinate wound-healing. Quiescent and activated dermal fibroblasts have similar metabolic rates, which suggests that hypometabolism is not necessarily associated with cellular quiescence (Valcourt et al., 2012). Unlike HSCs and lymphocytes, quiescent fibroblasts are not dependent on autophagy for survival and uptake glucose at rates comparable to proliferating fibroblasts (Valcourt et al., 2012). Multiple external cues, including contact inhibition and mitogen withdrawal, induce rat embryonic fibroblast to enter a non-proliferative quiescent state (Kwon et al., 2017). Fibroblasts that remain quiescent for longer move into a deeper quiescence and require greater stimulation or more time to reenter the cell cycle following serum stimulation (Kwon et al., 2017). As in organismal dormancy, quiescence in fibroblasts is heterogeneous and may entail a reactivation cost in deeply quiescent cells. However, unlike in organismal dormancy, deeply quiescent cells may remain metabolically active.

## QUIESCENCE AND DORMANCY IN CANCER CELLS

Mechanisms that regulate quiescence in normal cells provide insights into the pathways that promote quiescence in cancer cells. Cancer cells simply use or repurpose the processes, epigenetics, and genetics of normal cells. In normal cells, the trigger to divide or go quiescent is regulated by the availability of mitogenic growth factors, nutrients, and space. Loss of sensitivity to anti-growth signals is a hallmark of cancer (Hanahan and Weinberg, 2000) and a key aspect of tumorigenesis. Yet, the natural history of carcinogenesis and cancer eco-evolutionary dynamics do not always conform to continuous monotonic growth. The cancer cells making up a tumor live within a highly dynamic and interactive microenvironment consisting of fibroblasts, endothelial and inflammatory cells, growth factors, cytokines, vasculature, and lymph vessels. Collectively these constitute the tumor ecosystem. Fluctuations or interruptions in blood flow across a tumor or within regions of a tumor, lack of nutrients or space, or adverse interactions with normal cells can force cancer cells, and sometimes the tumor population as a whole, to pause rapid proliferation (Zahl et al., 2008; Almog, 2013; Hahnfeldt, 2013). Following treatment, a patient's cancer may remain clinically undetectable for months, years, and perhaps even decades (Aguirre-Ghiso, 2018). The disease seems to persist in a cell-arrested or non-proliferative state that is often referred to as "**dormancy**."

In 1934, Willis (1934) suggested "dormant" tumor cells as those that disseminated from the primary tumor and remain in a growth-arrested state. Twenty years later, Hadfield (1954) proposed that dormant tumor cells are in a temporary state of mitotic arrest. More recently, experimental models of dormancy have revealed that tumor dormancy may result from a balance between cell proliferation and death so that the tumor mass (i.e., small population of cancer cells) maintains a constant size. This balance may result from poor vascularization that limits nutrient availability to cells (Gimbrone et al., 1972; Wheelock et al., 1981) or from control by the immune system (Weinhold et al., 1979; Wheelock et al., 1981).

Cancer dormancy is thus divided into two categories (see **Figure 2**): (1) non-proliferative cancer cells persisting over a long period of time without dying (**cellular dormancy**) and (2) populations of cancer cells with cell proliferation balanced by cell death (**tumor mass dormancy**) (Enderling et al., 2012; Aguirre-Ghiso, 2007). Both of these categories may result in clinically undetectable cancer. The mechanisms producing them, however, are distinct and may present unique therapeutic opportunities. While dormant cancer cells are often assumed to have a lower metabolism, few studies have empirically quantified their metabolic activity (Endo et al., 2014; Carcereri de Prati et al., 2017). On the other hand, population-level dormancy includes metabolically active, proliferating cells. Because this concept of tumor population-level dormancy diverges from organismal dormancy, we do not further discuss it herein.

While the concept of cellular dormancy is often used in the context of disseminated cancer cells, drug-tolerant persisters and PACCs also exhibit a state that is stress resistant, quiescent, or dormant (Vallette et al., 2019; Pienta et al., 2020a). Because of their potential parallels to organismal dormancy, we consider these cells in our discussion below.

## Disseminated Tumor Cells

Disseminated tumor cells (DTCs) are cancer cells that have detached from the primary tumor and spread to other locations in the body through the circulatory system. DTCs may exist, undetected, in a non-proliferative state for extended periods, referred to as **cancer cell dormancy**. The microenvironment within the target organ in which the DTCs survive appears to play a critical role both in regulating the apparently **dormant** state of the DTCs, and their re-awakening into a proliferative state (Linde et al., 2016). Dormant DTCs may elude detection and attack by the adaptive immune system, and later be "reawakened by innate immune cells (neutrophils) responding to non-tumor inflammation" (Aguirre-Ghiso, 2018). Following reawakening, the active DTCs proliferate into metastases. Whether DTCs

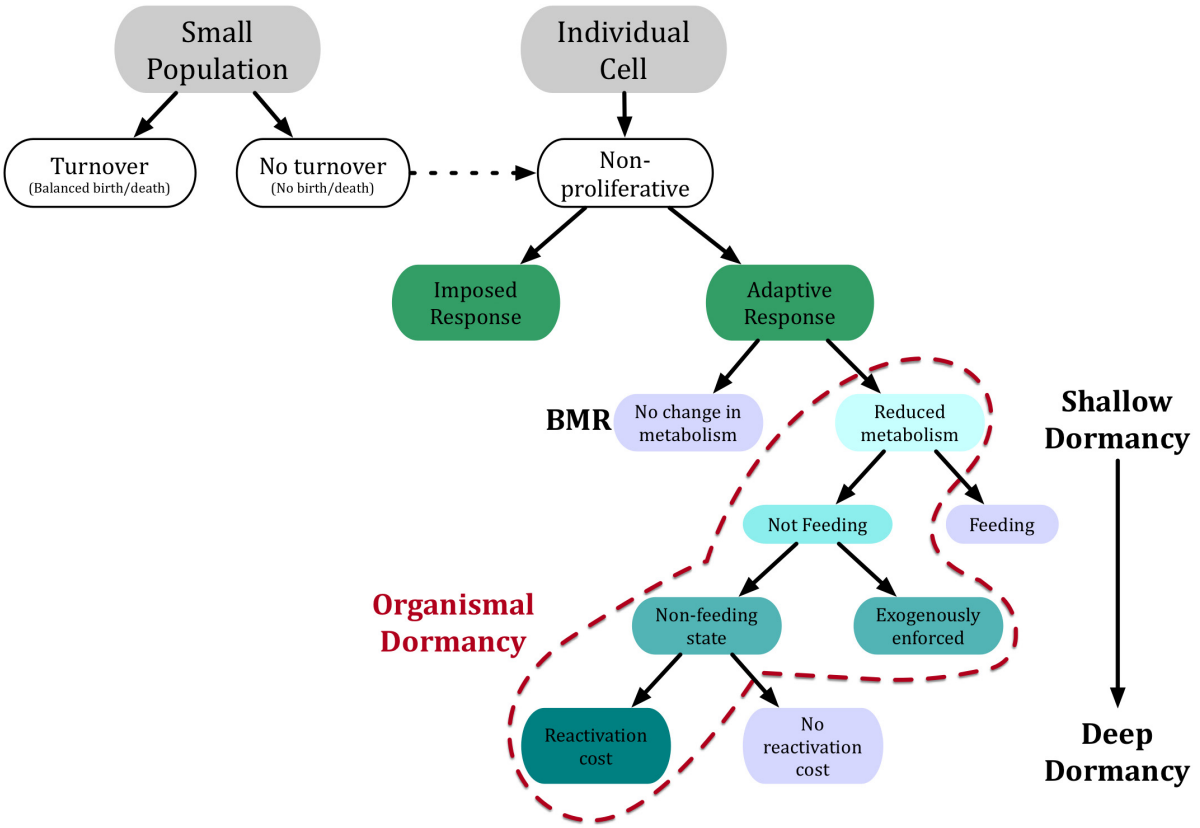


**FIGURE 2 |** Characteristics of cancer dormancy. Cancer dormancy describes either a **small population** of cells or **individual cell** dormancy. Small population level dormancy could either imply that the tumor mass maintains a constant size through balanced birth and death (**turnover**), or the tumor mass consists of a group of dormant cells (**no turnover**). Each dormant cell in the group may have the same characteristics of individual cell dormancy (black dotted arrow). **Imposed response** represents a form of dormancy that results from the absence of an environmental factor (a germination cue) required to break dormancy (e.g., the absence of oxygen in hypoxic environments, like waterlogged soils). Parallels between organismal dormancy and cancer dormancy exist at the individual level in which dormancy is an **adaptive response**. Organismal dormancy (red dashed ring) is a state characterized by **reduced metabolism**, **no feeding**, and a **reactivation cost**. In organisms, depth of dormancy increases as metabolic activity, body temperature, and sensitivity to growth signals decreases (deeper dormancy = darker teal). Cancer cells may have additional traits associated with quiescence or dormancy (purple boxes). **Exogenous factors** that prevent cancer cells from feeding include lack of resources or glucose transporters. The different aspects of dormancy depicted do not imply a series of physiological processes but the different depths of dormancy attained by different organisms.

detach early or late in the evolution of the primary tumor could also impact their potential to respond to dormancy cues, with late DTCs having greater metastatic growth potential either due to their later stage of evolution or the creation of pre-metastatic niches by early DTCs (Sosa et al., 2014). The processes of dissemination, dormancy, and reawakening may have critical clinical and therapeutic relevance.

The metastatic spread of cancer bears great resemblance to seed dispersal in plants or spores of microbes. The "seed and soil" hypothesis proposed by Paget (1889) suggests that the pattern of spread of the DTCs ("the seeds") within the body of a patient is due to the preferential growth and survival of DTCs within certain microenvironments ("the soil"), which could explain why particular cancers only metastasize in certain organs (e.g., prostate-to-bone). In plants, seeds (or microbial spores) disperse over short or long distances. Dispersed seeds can either immediately germinate or remain dormant. Delayed germination is beneficial to the plant. When seeds do not germinate at the same time they reduce competition, sib-sib competition, and spread the risk. Furthermore, dormancy and dispersal allow seeds to escape from unfavorable conditions or arrive at favorable conditions in time and space.

Similar to plant seeds, DTCs colonize microenvironments that may be favorable or unfavorable for growth. Like many cases of seed or spore dispersal, the vast majority of circulating tumor cells die and never become DTC's, but a tiny fraction may (Luzzi et al., 1998; Chambers et al., 2002; Lloyd et al., 2017). Microenvironments colonized by DTCs that are "non-permissive" for growth (e.g., hypoxic regions), activate stress signaling pathways that induce the DTC to enter **quiescence** (Aguirre-Ghiso, 2007). DTCs also colonize microenvironments where stem cells are found (stem cell niches) where signals that control HSC dormancy induce their dormancy. Because dormant HSCs can be found in hypoxic regions, these microenvironments may not be mutually exclusive (Lévesque et al., 2010). Although dormant DTCs are often assumed to be in a hypometabolic state, confirming this assumption is hampered by limited *in vivo* models. Insights into the metabolic state of the subset of DTCs located in hypoxic regions may be inferred from the metabolism of hypoxia resistant tumor cells.

## Hypoxia Resistant Tumor Cells

While DTCs may become dormant in certain regions of an organ that are hypoxic, cancer cells of actively growing tumors also experience hypoxia through temporal variations in intratumoral blood flow and instability of vasculature (Gillies et al., 2018). Although many tumor cells die in hypoxic environments, some survive by entering a state referred to as **dormancy**. Because few *in vitro* models of tumor cell dormancy exist, understanding the properties of these dormant cells is challenging. Existing evidence suggests that these cells undergo cell-cycle arrest in the G0/G1 phases or greatly reduce proliferation (Carcereri de Prati et al., 2017).

Hypoxia-resistant cells have a lower metabolism, as indicated by an 80% decrease in glucose consumption and lower pyruvate and lactate production (Endo et al., 2014; Carcereri de Prati et al., 2017). During chronic hypoxia, hypoxia-resistant cells upregulate autophagy to obtain nutrients despite the lower consumption of glucose (Carcereri de Prati et al., 2017). When hypoxia-resistant cells are reoxygenated, their proliferation rate returns to normal after a short delay. The reversibility of their decreased proliferation (Endo et al., 2014; Carcereri de Prati et al., 2017) thus requires metabolic activation and cell remodeling. Hypoxia-resistant cells are more resistant to chemotherapy, either because of their low proliferation rate or because the drug cannot reach the hypoxic regions of the tumor. While some cancer cells enter **quiescence** or proliferate more slowly under chronic hypoxia, cancer cells may utilize anaerobic glycolysis under acute hypoxia, increasing glucose uptake and lactate production (Endo et al., 2014). Hence, the duration of hypoxia influences the metabolic activity of cancer cells in hypoxic environments.

## Polyaneuploid Cancer Cells

Polyaneuploid cancer cells are aneuploid (have abnormal number of chromosomes) and undergo whole genome doubling in response to stress (Pienta et al., 2020a). These correspond to what others have described as polyploid giant cancer cells and persister cells (Illidge et al., 2000; Puig et al., 2008). They form from the fusion of 2N cells or from failed cytokinesis resulting in poly- or mono-nucleated polyaneuploids. This reversible state is also characterized by G0 cell cycle arrest (**quiescence**), increased cell size, and increased metabolic activity (distinguishing it from other quiescent states that are hypometabolic) (Pienta et al., 2020a). Once the stress is removed, and this can be months, PACCs re-enter the cell cycle and bud off non-polyploid (2N) progeny. They themselves do not proliferate as PACCs begetting PACCs. Such highly metabolic, resource uptaking, non-proliferative life history states have been found in a variety of taxa including bacteria (Valderrama et al., 2019), protists (Parfrey and Katz, 2010), fungi (Anderson et al., 2015), and plants and robustly permit survival to microenvironmental and therapeutic stress (Pienta et al., 2020a). It has been proposed that PACCs serve a decisive ecological role of allowing for increased storage, cell function, metabolic rate, and protection from stressors such as hypoxia, pH, metabolites, oxidative stress, and therapeutics. Evolutionarily, they increase heritable variation, permit self-genetic modification, and new functionality (see Table 1 from Pienta et al., 2021). The stress response in PACCs is reminiscent of organisms that undergo facultative sex such as *Daphnia magna* ("water fleas"), which can reproduce sexually and asexually based on environmental conditions. In good conditions, *D. magna* reproduce asexually to create clones, whereas under adverse conditions (e.g., cold or dry) they reproduce sexually to produce resting/diapausing eggs that are wrapped in a tough protective shell until conditions improve (Gerber et al., 2018). Sexual reproduction during adverse conditions permits genetic recombination, which, by increasing genetic diversity, may increase survival in a possibly altered environment following "awakening" from diapause.

## Drug-Tolerant Persisters

In cancer biology, "drug-tolerant persisters" refer to a subpopulation of cancer cells that are reversibly tolerant to treatment due to non-mutational mechanisms such as epigenetic

reprogramming (Sharma et al., 2010). This terminology is analogous to bacterial "persister cells," a small subset of antibacterial tolerant cells. Bacteria persisters form either stochastically or in response to antibiotic treatment, are slow dividing or growth arrested, and resume growth and drug sensitivity once the antibiotic is removed (Fisher et al., 2017). While there is no single definition of drug-tolerant persisters, four properties distinguish this state from cancer cell dormancy: (1) slow proliferation, (2) decreased sensitivity to treatment, (3) restoration of drug sensitivity and cell proliferation following treatment, and (4) contribution to genetic resistance (Shen S. et al., 2020). Slow-proliferation may not be sufficient for persister cells to survive therapy; evasion of therapy may also necessitate minimizing glucose consumption, changing their cell identity via the epithelial-to-mesenchymal transition, or interacting with other cell types in the tumor microenvironment (Shen S. et al., 2020). Most of these studies use 2D cultures, where drug-tolerant persisters are rare and result from stochastic epigenetic states (Sharma et al., 2010). However, recent studies suggest that in 3D cultures, treatment persistent residual tumors emerge and adopt a program similar to embryonic diapause, a reversible state of paused development in epiblasts that is triggered by adverse conditions (Dhimolea et al., 2021; Rehman et al., 2021). Similar to embryonic diapause, these "treatment persistent organoids" are characterized by quiescence or slow-cycling, downregulated metabolic and biosynthetic activity, increased cell adhesion, and increased autophagy (Dhimolea et al., 2021; Rehman et al., 2021). Thus, treatment persistent organoids may use an evolutionarily conserved mechanism that promotes survival under stress. An open research question concerns the degree to which persister cells in cancer and other microbial systems are polyaneuploid and vice-versa. Possible differences relate to whether these non-reproductive cells are polyploid or not, whether such cells are hyper- or hypo-metabolic, and whether such cells facilitate surviving unfavorable conditions as well as accelerating evolutionary changes such as drug resistance (Pienta et al., 2020b). For those studying these phenomena in yeast and bacteria, cancer may provide an ideal complementary experimental model organism (see section "Comparison of Dormancy in Other Organisms and in Cancer").

## Comparison of Dormancy in Other Organisms and in Cancer

Although direct parallels are few, many characteristics of quiescent cancer cells overlap with characteristics of dormant states in organisms (**Figure 2**). One similarity is that the duration of the response varies, with the longest duration associated with DTCs in cancer (months to decades) and endospores in organisms (centuries). While few studies in cancer have quantified some of the key traits of organismal dormancy including hypometabolism and reactivation cost, there is some evidence of these characteristics in hypoxia-resistant tumor cells (Endo et al., 2014; Carcereri de Prati et al., 2017). Like cysts and endospores, cancer cells may morphologically change in response to stress: hypoxia-resistant cells can have a longer shape and higher volume of cytoplasm compared to cells in non-resistant populations (Carcereri de Prati et al., 2017), drug-tolerant persisters may change their cell identity through epithelial-to-mesenchymal transition, and PACCs morph from a 2N state into a polyploid state (Pienta et al., 2020b).

On the other hand, even though hypoxia-resistant tumor cells have a lower metabolism, they may still be taking up and metabolizing nutrients since there is not a complete depletion of glucose consumption. This is in contrast to organisms, which are in a non-feeding state when they are dormant. Furthermore, some quiescent cancer cells such as PACCs may not be in a hypometabolic state. Lastly, in comparison to hibernators, whether cancer cells acquire and store energy prior to dormancy is not known, though they often rely on autophagy for survival (Carcereri de Prati et al., 2017). While the cardinal characteristic of organismal dormancy is hypometabolism, the primary feature of cancer cell dormancy is non-proliferation. Dormancy in cancer is only loosely associated with hypometabolism and lack of feeding, and the continuum of metabolic states in non-proliferative or slowly dividing cancer cells likely falls within a narrower range compared to organisms (**Figure 1**).

Yeast (*Saccharomyces* sp.) may provide some of the most direct comparisons between cancer cell and microbial cell dormancy (Hohmann and Mager, 2007). For instance when used for producing ethanol, the yeast must be able to tolerate and respond to temperature, oxidative, ethanol, and osmotic stressors (Saini et al., 2018). In response, yeast can exhibit the continuum of maintaining proliferation and activity under stress, reducing proliferation or switching to sexual reproduction (form haploid spores through meiosis that can combine to form the diploid state), changing metabolic state (i.e., via activation of heat shock proteins), forming PACC-like polyploids, or reducing metabolic activity and engaging in autophagy.

## Evolutionary Ecology of Dormancy Strategies

Variability in environmental conditions is common to all ecosystems, creating favorable and unfavorable periods for growth and survival. Natural ecosystems outside of cancer frequently experience temporal fluctuations in temperature and precipitation; in cancer, unpredictable patterns of blood flow cause temporal variations in nutrients, growth factors, pH, oxygen, and immune infiltration. Under these circumstances, dormancy is an adaptation that generally serves four possible functions: (1) bet-hedging, (2) avoiding over-crowding, (3) avoiding sib-sib competition, and (4) hunkering down and surviving unfavorable times (Simpson, 2007; Shefferson et al., 2018). The first three functions can select for a dormancy fraction where some of the population remain in an active state while others remain dormant. The fourth represents predictive dormancy where the organism or cancer cells have time to respond to the unfavorable conditions, and furthermore, can assess when conditions have improved. Cancer cells do exhibit predictive dormancy, may exhibit bet-hedging, and at present, there have been no experiments, to our knowledge, that would show whether cancer cells engage in dormancy to avoid over-crowding or sib-sib competition.

All of the above are in response to temporal variation. What of spatiotemporal variation? Venable and Brown (1988) used a mathematical model to predict how dormancy and dispersal traits in the seeds of annual plants co-adapt. As temporal autocorrelations decrease dormancy should be favored. In the absence of spatial autocorrelations and when spatial variability is on a smaller scale dispersal is favored over dormancy. Dormancy and dispersal can complement or substitute for each other, though not entirely. Snyder (2006) explores whether the presence of dormancy reduces the need for dispersal (Snyder, 2006). In terms of dispersal, cancer cells do exhibit stochastic and directed movement (chemotaxis) (Roussos et al., 2011; Sung and Weaver, 2017). But these movements, while large in relation to normal cells, are virtually sedentary compared to motile protists with cancer cells showing migration speeds of 0.4 μm per minute (Shen Q. et al., 2020), close to a body length per hour. This is one to three orders of magnitude slower than the speed of amoeboid cells (Van Haastert, 2011; Ildefonso et al., 2019). Dissemination in the blood as circulating tumor cells represents a long range dispersal that leads to highly improbable success. The extent to which cancers rely on dispersal as an adaptation to avoid spatiotemporal variability should be investigated in the context of dormancy. Regardless, dormancy should provide a prime adaptation for managing environmental uncertainty in cancer cells.

Most of our examples from nature and cancer have involved the use of predictive dormancy to respond to predictable or stochastic environmental variability. Ample evidence in terms of arrested cell cycles, PACCs, persister cells, and shifts toward autophagy show that cancer cells will cease proliferation and enter into some form of dormancy under harsh conditions. What of bet hedging? Bet-hedging is a strategy that evolves in unpredictably varying environments where expected fitness (arithmetic mean fitness) is sacrificed to reduce the temporal variance in fitness (geometric mean fitness) (Brockmann, 1987; Philippi and Seger, 1989) and was first proposed to explain why some plant seeds immediately germinate while others lie dormant (Cohen, 1966).

There can be "diversifying bet-hedging" where fractions of the population remain in different states regardless of current conditions. These states (e.g., fixed dormancy and germination fractions in annual plants) tradeoff fitness during good times and fitness during bad times (Childs et al., 2010; Starrfelt and Kokko, 2012). The bacteria *Bacillus subtilus*, like desert annuals, shows some stochastic sporulation regardless of nutrient conditions (Grimbergen et al., 2015). Similar examples can be found for the social amoeba *Dictyostelium discoideum* (Martínez-García and Tarnita, 2017), a marine amoeba *Flabellula baltica* (Fenchel, 2010), and in budding yeast *Saccharomyces cerevisiae* (Bagamery et al., 2020).

Diversifying bet-hedging in cancer does occur. PACCs have been identified as a small but ever-present fraction of the cancer cell population even in the absence of a major stress such as therapy, nutrient deprivation, or hypoxia (Lin et al., 2020). Cell culture experiments of dormancy show that during serum deprivation, a small proportion of cancer cells remained proliferative. Furthermore, when serum was replenished, a minor (but non-zero) proportion of cells remained non-proliferative (Barney et al., 2020). Some of these differences could arise due to the stochasticity in gene expression which generates phenotypic differences in cells that have the same genotype (Viney and Reece, 2013). Protein synthesis can promote rapid divergence so that sister cells are no more similar to each other than randomly chosen cell pairs (Spencer et al., 2009). Simons and Johnston (1997) suggest developmental instabilities as a source of diversifying bet-hedging, and the genetic instability of cancer cells may provide for bet-hedging through offspring with diverse heritable traits. Miller et al. (2020) explore how diversifying bet-hedging might promote coexistence of different cancer cell types as has been suggested for microbes (Jones and Lennon, 2010).

Conservative bet-hedging strategies involve a single state for the population where its trait value enhances survival under bad conditions while sacrificing opportunities during favorable times (Haaland et al., 2019, 2020). While most organisms' traits are a likely compromise between variable conditions, it can be hard to determine when such traits strictly tradeoff arithmetic with geometric mean fitness. Simons and Johnston (2003) provide an example with Indian tobacco *Lobelia inflate* where flowering time, while suboptimal in most years, serves well during bad years. Putative traits in cancer have not been studied in detail within the context of conservative bet-hedging. Pseudohypoxia has been proposed as such a case. Here, the cancer cells maintain high HIF-1 expression as a response to hypoxic conditions even under normoxia (Pressley et al., 2021). Other traits could include those associated with the maintenance of high levels of membrane pumps and metabolism to respond quickly to stochastic variation in the presence of toxins, low pH, damaging levels of oxygen free radicals, and even drug therapy. These traits are straying from dormancy *per se* but represent potentially critical forms of bet hedging. Dormancy strategies likely best fall into diversifying bet-hedging.

## DISCUSSION—FROM ECOLOGY TO CANCER AND BACK AGAIN

Cancer cells exist in a highly dynamic ecosystem where they experience both competition and cooperation with nearby cells. Increased understanding of the ecology and evolution of cancer is leading to new treatment strategies, like adaptive therapies that exploit cancer cell competition (Gatenby et al., 2009; Silva et al., 2012; Zhang et al., 2017). Similarly, better understanding dormancy from an ecological perspective may help devise new approaches to target these cells by exploiting the mechanisms that promote their awakening, maintenance, or eradication. Many advances have been made through interdisciplinary approaches between ecologists and cancer biologists. For such efforts to be fruitful, however, collaborators must possess a mutual understanding of technical terms such as "dormancy" and speak a common language. We believe that if the cancer biology community adopted a more precise definition of dormancy that also includes issues of reduced feeding, metabolism, robustness, stress tolerance, and reactivation costs rather than just lack of

proliferation, then it would ease the journey of ecologists trying to contribute to cancer research.

For the evolutionary ecologist interested in testing models and ideas pertaining to dormancy, cancer provides diverse experimental approaches. Cancer research can provide a rich spatial and temporal resolution of data not typically attainable in other natural or laboratory systems. In contrast to field studies, experiments can be replicated within the same cancer under a more controlled setting. Cancer biology may thus provide multiple avenues for testing both ecological and evolutionary theories of dormancy that are difficult to address otherwise. For example, cancer cell dormancy occurs *de novo* across a vast array of cancers, including multiple myeloma, prostate cancer metastasis to the bone, and breast cancer metastasis to the bone, lung, and brain (Phan and Croucher, 2020). This broad occurrence of dormancy could allow for the exploration of differences in how dormancy is regulated between cancers or metastatic sites, and whether those mechanisms resemble known regulation mechanisms found across taxa in organismal dormancy. Because cancers evolve rapidly, experimental cancer systems provide opportunities to test hypotheses regarding the environmental characteristics that select for the evolution of dormancy as an adaptation. Studying the evolution of cancer cell dormancy may provide novel insights into the evolution of dormancy at the organismal level.

Research on cancer brings an array of technologies for conducting experiments in mice (Lee et al., 2018), in organoids, as 3-D spheroids, or more traditional 2-D culture techniques. RNA sequencing (RNA-Seq) technologies permit measuring single cell expression of genes associated with cellular metabolism, proliferation, and cell membrane activity (Recasens and Munoz, 2019). Methylation profiling (Ferrer et al., 2020) and whole-genome or targeted genome sequencing can identify heritable differences between cancer cells within a cell line or between cell lines with diverse ecological properties (e.g., contrasts between breast cancer cells lines such as the highly glycolytic MDA-MB-231 and the non-glycolytic MCF-7). The Seahorse XF extracellular flux analyzer can measure single cell metabolism of cancer cells in different metabolic states, from different clones, or different cell lines (Bhatia et al., 2021). Immunohistochemical staining of cell cultures of histology preparations can identify metabolic markers of cell proliferation, metabolism, and other cellular properties that may be relevant to dormancy. Finally, selection experiments in the lab on cancer cell lines generally produce significant heritable changes within months. In this way, the technologies and resources available for cancer research may facilitate research on the evolutionary drivers of dormancy, just as understandings of dormancy in nature can add conceptual and terminological rigor to the insights gained from studying cancer dormancy.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

JB and CW contributed to the conception and design of the study. AM, CW, and JB wrote the manuscript. DB provided funding. All authors contributed to manuscript revisions, read, and approved the submitted version.

## FUNDING

DB and AM acknowledge funding from NCI (IMAG) U01CA202958 and PSON U01 (U01CA244101). JB acknowledges funding from NIH/NCI 1U54CA193489-01A1, Cancer as a Complex Adaptive System, NIH/NCI U54 Supplement, The tumor-host evolutionary arms race.

## ACKNOWLEDGMENTS

We thank Andriy Marusyk and Mostafa Nasr for helpful discussions related to the content of this manuscript, and the peer reviewers for their constructive feedback. **Figure 1** was created with ©BioRender—biorender.com as licensed by the Scientific Development Office of the Moffitt Cancer Center.

## REFERENCES

Aguirre-Ghiso, J. A. (2007). Models, mechanisms and clinical evidence for cancer dormancy. *Nat. Rev. Cancer* 7, 834–846. doi: 10.1038/nrc2256

Aguirre-Ghiso, J. A. (2018). How dormant cancer persists and reawakens. *Science* 361, 1314–1315. doi: 10.1126/science.aav0191

Almog, N. (2013). Genes and regulatory pathways involved in persistence of dormant micro-tumors. *Adv. Exp. Med. Biol.* 734, 3–17. doi: 10.1007/978-1-4614-1445-2_1

Anderson, C. A., Roberts, S., Zhang, H., Kelly, C. M., Kendall, A., Lee, C., et al. (2015). Ploidy variation in multinucleate cells changes under stress. *Mol. Biol. Cell* 26, 1129–1140. doi: 10.1091/mbc.e14-09-1375

Bagamery, L. E., Justman, Q. A., Garner, E. C., and Murray, A. W. (2020). A Putative Bet-hedging strategy buffers budding yeast against environmental instability. *Curr. Biol.* 30, 4563–4578. doi: 10.1016/j.cub.2020.08.092

Barnes, B. (1989). Freeze avoidance in a mammal: body temperatures below 0 degree C in an Arctic hibernator. *Science* 244, 1593–1595. doi: 10.1126/science.2740905

Barney, L. E., Hall, C. L., Schwartz, A. D., Parks, A. N., Sparages, C., Galarza, S., et al. (2020). Tumor cell-organized fibronectin maintenance of a dormant breast cancer population. *Sci. Adv.* 6:eaaz4157. doi: 10.1126/sciadv.aaz4157

Baskin, J. M., and Baskin, C. C. (2004). A classification system for seed dormancy. *Seed Sci. Res.* 14, 1–16. doi: 10.1079/SSR2003150

Bewley, J. D. (1997). Seed germination and dormancy. *Plant Cell* 96, 1055–1066. doi: 10.1105/tpc.9.7.1055

Bhatia, S., Thompson, E. W., and Gunter, J. H. (2021). "Studying the metabolism of epithelial-mesenchymal plasticity using the seahorse XFe96 extracellular flux analyzer," in *The Epithelial-to Mesenchymal Transition*, eds K. Campbell and E. Theveneau (Berlin: Springer), 327–340. doi: 10.1007/978-1-0716-0779-4_25

Brockmann, S. (1987). What is bet-hedging. *Oxford Surv. Evol. Biol.* 4, 182–211.

Bryder, D., Rossi, D. J., and Weissman, I. L. (2006). Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am. J. Pathol.* 169, 338–346. doi: 10.2353/ajpath.2006.060312

Carcereri de Prati, A., Butturini, E., Rigo, A., Oppici, E., Rossin, M., Boriero, D., et al. (2017). Metastatic breast cancer cells enter into dormant state and express cancer stem cells phenotype under chronic hypoxia. *J. Cell. Biochem.* 118, 3237–3248. doi: 10.1002/jcb.25972

Chambers, A. F., Groom, A. C., and MacDonald, I. C. (2002). Dissemination and growth of cancer cells in metastatic sites. *Nat. Rev. Cancer* 2, 563–572. doi: 10.1038/nrc865

Childs, D. Z., Metcalf, C. J. E., and Rees, M. (2010). Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. *Proc. R. Soc. B Biol. Sci.* 277, 3055–3064. doi: 10.1098/rspb.2010.0707

Cohen, D. (1966). Optimizing reproduction in a randomly varying environment. *J. Theor. Biol.* 12, 119–129. doi: 10.1016/0022-5193(66)90188-3

Corliss, J. O. (ed.) (2001). "Protozoan cysts and spores," in *eLS* (Hoboken, NJ: Wiley).

Denlinger, D. L. (2009). "Diapause," in *Encyclopedia of insects*, eds V. Resh and R. Carde (Amsterdam: Elsevier), 267–271.

Dhimolea, E., de Matos Simoes, R., Kansara, D., Al'Khafaji, A., Bouyssou, J., Weng, X., et al. (2021). An embryonic diapause-like adaptation with suppressed myc activity enables tumor treatment persistence. *Cancer Cell* 39, 240.e11–256.e11. doi: 10.1016/j.ccell.2020.12.002

Ellegaard, M., and Ribeiro, S. (2018). The long-term persistence of phytoplankton resting stages in aquatic 'seed banks.'. *Biol. Rev.* 93, 166–183. doi: 10.1111/brv.12338

Enderling, H., Almog, N., and Hlatky, L. (2012). *Systems Biology of Tumor Dormancy*. Berlin: Springer Science & Business Media.

Endo, H., Okuyama, H., Ohue, M., and Inoue, M. (2014). Dormancy of cancer cells with suppression of AKT activity contributes to survival in chronic hypoxia. *PLoS One* 9:e0098858. doi: 10.1371/journal.pone.0098858

Fenchel, T. (2010). The life history of *Flabellula baltica* Smirnov (Gymnamoebae, Rhizopoda): adaptations to a spatially and temporally heterogeneous environment. *Protist* 161, 279–287. doi: 10.1016/j.protis.2009.10.005

Ferrer, A. I., Trinidad, J. R., Sandiford, O., Etchegaray, J.-P., and Rameshwar, P. (2020). Epigenetic dynamics in cancer stem cell dormancy. *Cancer Metastasis Rev.* 39, 721–738. doi: 10.1007/s10555-020-09882-x

Fiore, A. P. Z. P., Ribeiro, P., de, F., and Bruni-Cardoso, A. (2018). Sleeping beauty and the microenvironment enchantment: microenvironmental regulation of the proliferation-quiescence decision in normal tissues and in cancer development. *Front. Cell Dev. Biol.* 6:59. doi: 10.3389/fcell.2018.00059

Fisher, R. A., Gollan, B., and Helaine, S. (2017). Persistent bacterial infections and persister cells. *Nat. Rev. Microbiol.* 15:453. doi: 10.1038/nrmicro.2017.42

Gatenby, R. A., Silva, A. S., Gillies, R. J., and Frieden, B. R. (2009). Adaptive therapy. *Cancer Res.* 69, 4894–4903.

Gerber, N., Kokko, H., Ebert, D., and Booksmythe, I. (2018). Daphnia invest in sexual reproduction when its relative costs are reduced. *Proc. R. Soc. B Biol. Sci.* 285:20172176. doi: 10.1098/rspb.2017.2176

Gilbert, J. J. (1974). Dormancy in rotifers. *Trans. Am. Microsc. Soc.* 93, 490–513. doi: 10.2307/3225154

Gillies, R. J., Brown, J. S., Anderson, A. R. A., and Gatenby, R. A. (2018). Eco-evolutionary causes and consequences of temporal changes in intratumoural blood flow. *Nat. Rev. Cancer* 18, 576–585. doi: 10.1038/s41568-018-0030-7

Gimbrone, M. A. Jr., Leapman, S. B., Cotran, R. S., and Folkman, J. (1972). Tumor dormancy in vivo by prevention of neovascularization. *J. Exp. Med.* 136, 261–276. doi: 10.1084/jem.136.2.261

Grimbergen, A. J., Siebring, J., Solopova, A., and Kuipers, O. P. (2015). Microbial bet-hedging: the power of being different. *Curr. Opin. Microbiol.* 25, 67–72. doi: 10.1016/j.mib.2015.04.008

Haaland, T. R., Wright, J., and Ratikainen, I. I. (2020). Generalists versus specialists in fluctuating environments: a bet-hedging perspective. *Oikos* 129, 879–890. doi: 10.1111/oik.07109

Haaland, T. R., Wright, J., Tufto, J., and Ratikainen, I. I. (2019). Short-term insurance versus long-term bet-hedging strategies as adaptations to variable environments. *Evolution* 73, 145–157. doi: 10.1111/evo.13659

Hadfield, G. (1954). The dormant cancer cell. *Br. Med. J.* 2:607. doi: 10.1136/bmj.2.4888.607

Hahnfeldt, P. (2013). "The host support niche as a control point for tumor dormancy: implications for tumor development and beyond," in *Systems Biology of Tumor Dormancy*, eds H. Enderling, N. Almog, and L. Hlatky (Berlin: Springer), 19–35. doi: 10.1007/978-1-4614-1445-2_2

Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* 100, 57–70.

Hohmann, S., and Mager, W. H. (2007). *Yeast Stress Responses*. Berlin: Springer Science & Business Media.

Ildefonso, M., Bringas, C., Malaina, I., Regner, B., Pérez-Samartín, A., Boyano, M. D., et al. (2019). The nucleus does not significantly affect the migratory trajectories of amoeba in two-dimensional environments. *Sci. Rep.* 9, 1–15.

Illidge, T. M., Cragg, M. S., Fringes, B., Olive, P., and Erenpreisa, J. A. (2000). Polyploid giant cells provide a survival mechanism for p53 mutant cells after DNA damage. *Cell Biol. Int.* 24, 621–633. doi: 10.1006/cbir.2000.0557

Jones, S. E., and Lennon, J. T. (2010). Dormancy contributes to the maintenance of microbial diversity. *Proc. Natl. Acad. Sci.* 107, 5881–5886. doi: 10.1073/pnas.0912765107

Kwon, J. S., Everetts, N. J., Wang, X., Wang, W., Della Croce, K., Xing, J., et al. (2017). Controlling depth of cellular quiescence by an Rb-E2F network switch. *Cell Rep.* 20, 3223–3235. doi: 10.1016/j.celrep.2017.09.007

Lee, R. E. (2009). "Chapter 79 - Dormancy," in *Encyclopedia of insects*, eds V. Resh and R. Carde (San Diego: Academic Press), 300–301.

Lee, S. H., Park, S. A., Zou, Y., Seo, S.-U., Jun, C.-D., Lee, W. J., et al. (2018). Real-time monitoring of cancer cells in live mouse bone marrow. *Front. Immunol.* 9:1681. doi: 10.3389/fimmu.2018.01681

Lévesque, J. P., Helwani, F. M., and Winkler, I. G. (2010). The endosteal 'osteoblastic' niche and its role in hematopoietic stem cell homing and mobilization. *Leukemia* 24, 1979–1992. doi: 10.1038/leu.2010.214

Li, N., and Clevers, H. (2010). Coexistence of quiescent and active adult stem cells in mammals. *Science* 327, 542–545. doi: 10.1126/science.1180794

Lin, K.-C., Sun, Y., Torga, G., Sherpa, P., Zhao, Y., Qu, J., et al. (2020). An in vitro tumor swamp model of heterogeneous cellular and chemotherapeutic landscapes. *Lab. Chip* 20, 2453–2464. doi: 10.1039/d0lc00131g

Linde, N., Fluegen, G., and Aguirre-Ghiso, J. A. (2016). The relationship between dormant cancer cells and their microenvironment. *Adv. Cancer Res.* 132, 45–71. doi: 10.1016/bs.acr.2016.07.002

Lloyd, M. C., Gatenby, R. A., and Brown, J. S. (2017). "Ecology of the metastatic process," in *Ecology and Evolution of Cancer*, eds B. Ujvari, B. Roche, and F. Thomas (Amsterdam: Elsevier), 153–165. doi: 10.1016/b978-0-12-804310-3.00011-9

Luzzi, K. J., MacDonald, I. C., Schmidt, E. E., Kerkvliet, N., Morris, V. L., Chambers, A. F., et al. (1998). Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *Am. J. Pathol.* 153, 865–873.

Lyman, C. P., Willis, J. S., Malan, A., and Wang, L. C. H. (1982). *Hibernation and Torpor in Birds and Mammals*. New York, NY: Aca-demic Press.

Martin, S. L., and Yoder, A. D. (2014). Theme and variations: heterothermy in mammals. *Am. Zool.* 54, 439–442. doi: 10.1093/icb/icu085

Martínez-García, R., and Tarnita, C. E. (2017). Seasonality can induce coexistence of multiple bet-hedging strategies in Dictyostelium discoideum via storage effect. *J. Theor. Biol.* 426, 104–116. doi: 10.1016/j.jtbi.2017.05.019

Masaki, S. (2009). "Aestivation," in *Encyclopedia of Insects*, eds V. H. Resh and R. T. Cardé (Amsterdam: Elsevier), 2–4.

Melvin, R. G., and Andrews, M. T. (2009). Torpor induction in mammals: recent discoveries fueling new ideas. *Trends Endocrinol. Metab.* 20, 490–498. doi: 10.1016/j.tem.2009.09.005

Miller, A. K., Brown, J. S., Basanta, D., and Huntly, N. (2020). What is the storage effect, why should it occur in cancers, and how can it inform cancer therapy? *Cancer Control* 27, 1–9. doi: 10.1177/1073274820941968

Mury, S. P., and Popham, D. L. (2014). Bacterial endospores. *eLS* 26:300.

Paget, S. (1889). The distribution of secondary growths in cancer of the breast. *Lancet* 133, 571–573. doi: 10.1016/s0140-6736(00)49915-0

Parfrey, L. W., and Katz, L. A. (2010). Genome dynamics are influenced by food source in Allogromia laticollaris strain CSH (Foraminifera). *Genome Biol. Evol.* 2, 678–685. doi: 10.1093/gbe/evq051

Phan, T. G., and Croucher, P. I. (2020). The dormant cancer cell life cycle. *Nat. Rev. Cancer* 20, 398–411. doi: 10.1038/s41568-020-0263-0

Philippi, T., and Seger, J. (1989). Hedging one's evolutionary bets, revisited. *Trends Ecol. Evol.* 4, 41–44. doi: 10.1016/0169-5347(89)90138-9

Pienta, K. J., Hammarlund, E. U., Austin, R. H., Axelrod, R., Brown, J. S., and Amend, S. R. (2020a). Cancer cells employ an evolutionarily conserved polyploidization program to resist therapy. *Semin. Cancer Biol.* [Epub ahead of print].

Pienta, K. J., Hammarlund, E. U., Axelrod, R., Amend, S. R., and Brown, J. S. (2020b). Convergent evolution, evolving evolvability, and the origins of lethal cancer. *Mol. Cancer Res.* 18, 801–810. doi: 10.1158/1541-7786.MCR-19-1158

Pienta, K. J., Hammarlund, E. U., Brown, J. S., Amend, S. R., and Axelrod, R. M. (2021). Cancer recurrence and lethality are enabled by enhanced survival and reversible cell cycle arrest of polyaneuploid cells. *Proc. Natl. Acad. Sci. U.S.A.* 118:e2020838118. doi: 10.1073/pnas.2020838118

Pressley, M., Gallaher, J. A., Brown, J. S., Tomaszewski, M. R., Borad, P., Damaghi, M., et al. (2021). Cycling hypoxia selects for constitutive HIF stabilization. *Sci. Rep.* 11, 1–14.

Puig, P., Guilly, M., Bouchot, A., Droin, N., Cathelin, D., Bouyer, F., et al. (2008). Tumor cells can escape DNA-damaging cisplatin through DNA endoreduplication and reversible polyploidy. *Cell Biol. Int.* 32, 1031–1043. doi: 10.1016/j.cellbi.2008.04.021

Recasens, A., and Munoz, L. (2019). Targeting Cancer Cell Dormancy. *Trends Pharmacol. Sci.* 40, 128–141. doi: 10.1016/j.tips.2018.12.004

Rehman, S. K., Haynes, J., Collignon, E., Goyal, S., Moffat, J., and Brien, C. A. O. (2021). Colorectal cancer cells enter a diapause-like DTP state to survive chemotherapy. *Cell* 184, 226.e21–242.e21. doi: 10.1016/j.cell.2020.11.018

Ribeiro, S., Berge, T., Lundholm, N., Andersen, T. J., Abrantes, F., and Ellegaard, M. (2011). Phytoplankton growth after a century of dormancy illuminates past resilience to catastrophic darkness. *Nat. Commun.* 2:311. doi: 10.1038/ncomms1314

Ricci, C. (2001). Dormancy patterns in rotifers. *Hydrobiologia* 446, 1–11. doi: 10.1007/978-94-010-0756-6_1

Rohde, A., and Bhalerao, R. P. (2007). Plant dormancy in the perennial context. *Trends Plant Sci.* 12, 217–223. doi: 10.1016/j.tplants.2007.03.012

Ross, B. J., and Hallock, P. (2016). Dormancy in the foraminifera: a review. *J. Foraminifer. Res.* 46, 358–368. doi: 10.2113/gsjfr.46.4.358

Roussos, E. T., Condeelis, J. S., and Patsialou, A. (2011). Chemotaxis in cancer. *Nat. Rev. Cancer* 11, 573–587. doi: 10.1038/nrc3078

Sadoff, H. L. (1975). Encystment and germination in *Azotobacter vinelandii*. *Bacteriol. Rev.* 39, 516–539. doi: 10.1128/mmbr.39.4.516-539.1975

Saini, P., Beniwal, A., Kokkiligadda, A., and Vij, S. (2018). Response and tolerance of yeast to changing environmental stress during ethanol fermentation. *Process Biochem.* 72, 1–12. doi: 10.1016/j.procbio.2018.07.001

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*. Cambridge, MA: Cambridge university press.

Sharma, S. V., Lee, D. Y., Li, B., Quinlan, M. P., Takahashi, F., Maheswaran, S., et al. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* 141, 69–80. doi: 10.1016/j.cell.2010.02.027

Shefferson, R. P., Kull, T., Hutchings, M. J., Selosse, M., Jacquemyn, H., Kellett, K. M., et al. (2018). Drivers of vegetative dormancy across herbaceous perennial plant species. *Ecol. Lett.* 21, 724–733. doi: 10.1111/ele.12940

Shen, Q., Niederstrasser, H., Barakat, R., Haddadin, Z., Miller, S. R., Posner, B., et al. (2020). Single-cell-level screening method for migratory cancer cells and its potential feasibility in high-throughput manner. *Biofabrication* 12:35019.

Shen, S., Vagner, S., and Robert, C. (2020). Persistent cancer cells: the deadly survivors. *Cell* 183, 860–874. doi: 10.1016/j.cell.2020.10.027

Silva, A. S., Kam, Y., Khin, Z. P., Minton, S. E., Gillies, R. J., and Gatenby, R. A. (2012). Evolutionary approaches to prolong progression-free survival in breast cancer. *Cancer Res.* 72, 6362–6370. doi: 10.1158/0008-5472.can-12-2235

Simons, A. M., and Johnston, M. O. (1997). Developmental instability as a bet-hedging strategy. *Oikos* 80, 401–406. doi: 10.2307/3546608

Simons, A. M., and Johnston, M. O. (2003). Suboptimal timing of reproduction in *Lobelia inflata* may be a conservative bet-hedging strategy. *J. Evol. Biol.* 16, 233–243. doi: 10.1046/j.1420-9101.2003.00530.x

Simpson, G. M. (2007). *Seed Dormancy in Grasses*. Cambridge, MA: Cambridge University Press.

Snyder, R. E. (2006). Multiple risk reduction mechanisms: can dormancy substitute for dispersal? *Ecol. Lett.* 9, 1106–1114. doi: 10.1111/j.1461-0248.2006.00962.x

Sosa, M. S., Bragado, P., and Aguirre-Ghiso, J. A. (2014). Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat. Rev. Cancer* 14, 611–622. doi: 10.1038/nrc3793

Spencer, S. L., Gaudet, S., Albeck, J. G., Burke, J. M., and Sorger, P. K. (2009). Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature* 459, 428–432. doi: 10.1038/nature08012

Starrfelt, J., and Kokko, H. (2012). Bet-hedging-a triple trade-off between means, variances and correlations. *Biol. Rev.* 87, 742–755. doi: 10.1111/j.1469-185X.2012.00225.x

Sung, B. H., and Weaver, A. M. (2017). Exosome secretion promotes chemotaxis of cancer cells. *Cell Adh. Migr.* 11, 187–195. doi: 10.1080/19336918.2016.1273307

Valcourt, J. R., Lemons, J. M. S., Haley, E. M., Kojima, M., Demuren, O. O., and Coller, H. A. (2012). Staying alive: metabolic adaptations to quiescence. *Cell Cycle* 11, 1680–1696. doi: 10.4161/cc.19879

Valderrama, J. A., Kulkarni, S. S., Nizet, V., and Bier, E. (2019). A bacterial gene-drive system efficiently edits and inactivates a high copy number antibiotic resistance locus. *Nat. Commun.* 10, 1–8.

Vallette, F. M., Olivier, C., Lézot, F., Oliver, L., Cochonneau, D., Lalier, L., et al. (2019). Dormant, quiescent, tolerant and persister cells: four synonyms for the same target in cancer. *Biochem. Pharmacol.* 162, 169–176. doi: 10.1016/j.bcp.2018.11.004

Van Haastert, P. J. M. (2011). Amoeboid cells use protrusions for walking, gliding and swimming. *PLoS One* 6:e27532. doi: 10.1371/journal.pone.0027532

Venable, D. L., and Brown, J. S. (1988). The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *Am. Nat.* 131, 360–384. doi: 10.1086/284795

Villanueva-Cañas, J. L., Faherty, S. L., Yoder, A. D., and Albà, M. M. (2014). Comparative genomics of mammalian hibernators using gene networks. *Integr. Comp. Biol.* 54, 452–462. doi: 10.1093/icb/icu048

Viney, M., and Reece, S. E. (2013). Adaptive noise. *Proc. R. Soc. B Biol. Sci.* 280:3. doi: 10.1098/rspb.2013.1104

Weinhold, K. J., Goldstein, L. T., and Wheelock, E. F. (1979). The tumor dormant state. Quantitation of L5178Y cells and host immune responses during the establishment and course of dormancy in syngeneic DBA/2 mice. *J. Exp. Med.* 149, 732–744. doi: 10.1084/jem.149.3.732

Wheelock, E. F., Weinhold, K. J., and Levich, J. (1981). The tumor dormant state. *Adv. Cancer Res.* 34, 107–140. doi: 10.1016/s0065-230x(08)60240-7

Willis, R. A. (1934). *The Spread of Tumours in the Human Body*. London: J. & A. Churchill.

Wilson, A., Laurenti, E., Oser, G., van der Wath, R. C., Blanco-Bose, W., Jaworski, M., et al. (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* 135, 1118–1129. doi: 10.1016/j.cell.2008.10.048

Wilsterman, K., Ballinger, M. A., and Williams, C. M. (2020). A unifying, eco-physiological framework for animal dormancy. *Funct. Ecol.* 35, 11–31. doi: 10.1111/1365-2435.13718

Withers, P. C., and Cooper, C. E. (2010). Metabolic depression: a historical perspective. *Prog. Mol. Subcell Biol.* 49, 1–23. doi: 10.1007/978-3-642-02421-4_1

Yao, G. (2014). Modelling mammalian cellular quiescence. *Interface Focus* 4:20130074. doi: 10.1098/rsfs.2013.0074

Zahl, P.-H., Mæhlen, J., and Welch, H. G. (2008). The natural history of invasive breast cancers detected by screening mammography. *Arch. Intern. Med.* 168, 2311–2316. doi: 10.1001/archinte.168.21.2311

Zhang, J., Cunningham, J. J., Brown, J. S., and Gatenby, R. A. (2017). Integrating evolutionary dynamics into treatment of metastatic castrate-resistant prostate cancer. *Nat. Commun.* 8, 1–9.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 20 July 2021
doi: 10.3389/fevo.2021.676071

# Coupled Source-Sink Habitats Produce Spatial and Temporal Variation of Cancer Cell Molecular Properties as an Alternative to Branched Clonal Evolution and Stem Cell Paradigms

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**Specialty section:**
This article was submitted to Models in Ecology and Evolution, a section of the journal Frontiers in Ecology and Evolution

**Received:** 04 March 2021
**Accepted:** 28 June 2021
**Published:** 20 July 2021

**Citation:**
Cunningham JJ, Bukkuri A, Brown JS, Gillies RJ and Gatenby RA (2021) Coupled Source-Sink Habitats Produce Spatial and Temporal Variation of Cancer Cell Molecular Properties as an Alternative to Branched Clonal Evolution and Stem Cell Paradigms. Front. Ecol. Evol. 9:676071. doi: 10.3389/fevo.2021.676071

Intratumoral molecular cancer cell heterogeneity is conventionally ascribed to the accumulation of random mutations that occasionally generate fitter phenotypes. This model is built upon the "mutation-selection" paradigm in which mutations drive ever-fitter cancer cells independent of environmental circumstances. An alternative model posits spatio-temporal variation (e.g., blood flow heterogeneity) drives speciation by selecting for cancer cells adapted to each different environment. Here, spatial genetic variation is the consequence rather than the cause of intratumoral evolution. In nature, spatially heterogenous environments are frequently coupled through migration. Drawing from ecological models, we investigate adjacent well-perfused and poorly-perfused tumor regions as "source" and "sink" habitats, respectively. The source habitat has a high carrying capacity resulting in more emigration than immigration. Sink habitats may support a small ("soft-sink") or no ("hard-sink") local population. Ecologically, sink habitats can reduce the population size of the source habitat so that, for example, the density of cancer cells directly around blood vessels may be lower than expected. Evolutionarily, sink habitats can exert a selective pressure favoring traits different from those in the source habitat so that, for example, cancer cells adjacent to blood vessels may be suboptimally adapted for that habitat. Soft sinks favor a generalist cancer cell type that moves between the environment but can, under some circumstances, produce speciation events forming source and sink habitat specialists resulting in significant molecular variation in cancer cells separated by small distances. Finally, sink habitats, with limited blood supply, may receive reduced concentrations of systemic drug treatments; and local hypoxia and acidosis may further decrease drug efficacy allowing cells to survive treatment and evolve resistance. In such cases, the sink transforms into the source habitat for resistant cancer cells, leading to treatment failure

and tumor progression. We note these dynamics will result in spatial variations in molecular properties as an alternative to the conventional branched evolution model and will result in cellular migration as well as variation in cancer cell phenotype and proliferation currently described by the stem cell paradigm.

**Keywords: cancer heterogeneity, cancer vascularity, branching clonal evolution, source-sink habitats, cancer ecology, cancer evolution**

## INTRODUCTION

Regional variations in the molecular properties of cancer cells have been well established and are usually ascribed to accumulation of genetic changes, often called branched evolution, as each mutation initiates a new species (Fisher et al., 2012; Gerlinger et al., 2012; Zhang et al., 2019). This conceptual model is built upon the gene centric view of evolution, summarized as "mutation-selection," in which cancer cells experience random mutations at a rate higher than normal cells and each mutation is then subject to selection by the overall tumor environment. Though most mutations are deleterious, the rare mutation that increases fitness will allow increased proliferation producing a genetically distinct subpopulation and, therefore, observable regional genotypic variations.

However, this paradigm (Archetti, 2013; Scott and Marusyk, 2017; Hinohara and Polyak, 2019) tends to neglect the role of spatio-temporal heterogeneity in environmental selection forces as a driver of evolution. In general, the fitness of any cancer cell is defined by the interaction of its phenotype with local environmental conditions. As conditions change in space so will the optimal phenotype of the cancer cells. Thus, natural selection may favor genetically and molecularly distinct cancer cells phenotypically suited to the local habitat type. But, these local habitat-specific cancer cell populations are not completely isolated. They are connected and more or less coupled through migration, the dispersal of individuals between habitats (**Figure 1**). Here we explore migration as a previously unrecognized driver of intra-tumoral evolution (Winker, 2000).

Initially described by Pulliam (1988), local movement of individuals can connect adjacent habitats with very different properties. For example, a "source habitat" has favorable environmental conditions and, therefore, a positive per capita population growth rate. Within tumors, a source habitat might be one that is well perfused with a large carrying capacity. In contrast, a "sink habitat" has unfavorable environmental conditions in which net mortality exceeds reproduction resulting in a higher within-habitat death than birth rate. In tumors, this would correspond to a region with little or no blood flow resulting in environmental conditions that, in the absence of migration, supports few if any cancer cells. When physically adjacent, these disparate habitats can be coupled through migration; and, within these metapopulations, a large fraction of individuals may reside in habitats that are, in the absence of migration, insufficient to maintain a net positive growth rate. Furthermore, consistent movement between habitats may alter the evolution of cancer cell phenotype resulting in habitat specialization or a single generalist cancer cell type whose adaptations balance exposure to both habitats (Holt and Gomulkiewicz, 1997).

In nature, it has been demonstrated, both theoretically (Brown and Pavlovic, 1992) and empirically (Boughton, 1999), that source-sink dynamics can act both spatially (Holt, 1985) and temporally (Johnson, 2004) to profoundly influence regional metapopulations residing in and moving between different habitats (Gravel et al., 2010). In particular, migration between habitats can result in speciation and subsequent co-existence of multiple different species. Thus, in addition to mutation, genetic drift and natural selection, evolutionary ecologists have come to recognize migration as a significant evolutionary force (Brown and Pavlovic, 1992). As noted by Brown and Pavlovic (1992) "when viewed as a property of the environment rather than a force of evolution, migration becomes part of the circumstances to which evolution by natural selection responds."

Within tumors, the ability of individual cells to migrate (typically $\sim$ 5 to 10 $\mu$m/h) is recognized as a critical phenotypic adaptation for survival and cancer progression (Yamaguchi et al., 2005; Polacheck et al., 2013; Te Boekhorst et al., 2016; Paul et al., 2017; Staneva et al., 2019)). Migration is typically associated with epithelial-to-mesenchymal transition, wherein the latter phenotype is motile (Dongre and Weinberg, 2019). Once it arrives at a novel location or tissue, the cell can undergo the reverse: a mesenchymal to epithelial transition. Furthermore, the cancer stem-cell paradigm (Li et al., 2007; Walcher et al., 2020; Wang et al., 2020) posits a stem cell niche from which non-stem cells migrate (i.e., phenotypically distinct and not self-replicating) into adjacent tumor regions (Borovski et al., 2011). Here we note that "stem cells" may indeed be cells that occupy a source habitat and migration of these cells into a sink habitat produces both the phenotypic variation and reduced proliferative capacity described in the stem cell paradigm.

The specific source-sink dynamics depend highly on the characteristics of the sink environment. A black-hole or hard-sink habitat cannot sustain a viable population in the absence of continued immigration. Regardless of population size, in a hard sink, the individuals will experience a negative per capita growth rate. Within tumors, this would correspond to a region with little or no blood flow resulting in environmental conditions with a carrying capacity that is near zero. Migration from the source habitat can maintain a population within the sink habitat. The existence of multiple microscopic clusters of viable cells within macroscopic "necrotic" areas of tumors is well known in pathology (Jardim-Perassi et al., 2019). Hard sink habitats can provide some return migrants to the source habitat, influencing evolution, and even ecologically rescuing a source habitat from a catastrophic perturbation (Holt et al., 2004).

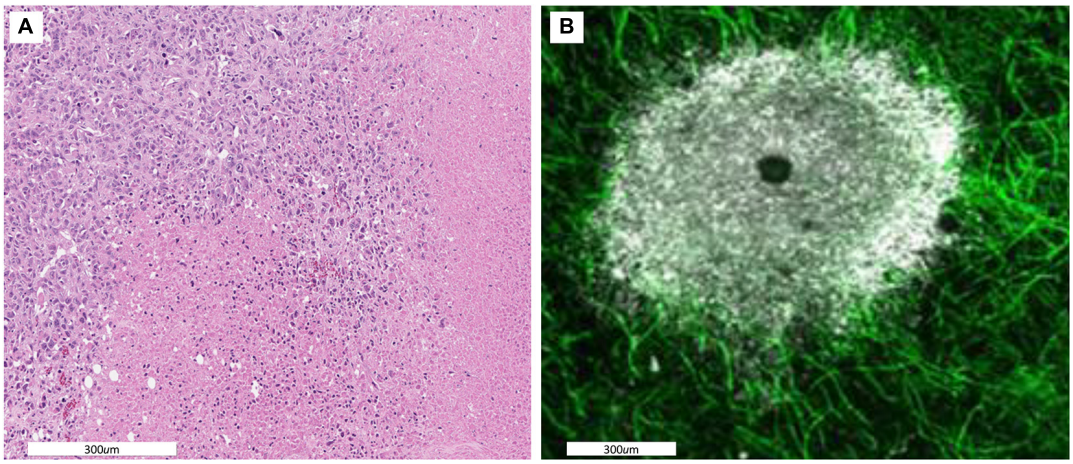


**FIGURE 1 | (A)** A histological section showing spatial variations in intratumoral habitats. Cellular density is high in the upper left indicating a well perfused tumor region. The lower and right side of the images shows regions in which most cells are necrotic indicating little perfusion. **(B)** A dorsal window chamber view of a tumor grown in a mouse expressing endothelial GFP. Tumor cells are shown in white. As with the histological staining there is a clear well perfused vascular edge with a less dense avascular internal region and a necrotic core. Migration rates of $\sim$ 5 to 10 $\mu$m/h allow for individual cells to traverse within and between these habitats.

Alternatively, the less favorable habitat may act as a "soft" sink, which can support a viable population, albeit one that is much smaller than the source habitat. Asymmetries in population sizes or migration rates means that more individuals move from the richer habitat to the poorer than vice-versa. Under density dependent population growth, this means the system equilibrates to a steady state in which the source habitat is underpopulated (below its carrying capacity) and the sink habitat is overpopulated (above its carrying capacity). Source and sink habitats may exert selection for quite different phenotypic and genotypic properties; so much so that there is a potential for speciation and diversification (Cure et al., 2017).

We propose source-sink dynamics contribute to the spatial variability in molecular properties of cancer cells observed within and between tumors in the same patient. Some regions of a tumor and regions of the body represent hard sinks in which a dispersing cancer cell faces near immediate death upon arrival. For example, circulating tumor cells may extravasate (exit the circulating system) into a tissue totally unsuitable for survival so that a metastases never forms. Within the tumor, necrotic zones provide a hard sink. Examples of soft sink habitats may include poorly vascularized tumor regions or perhaps inflamed but otherwise normal tissue at the tumor edge.

Here, we illustrate the eco-evolutionary dynamics associated with source sink dynamics in black-hole, hard- and soft-sink circumstances. We focus on how migration into a sink habitat influences 1) local and total population sizes, 2) possible extinction of the entire population, 3) evolution of a trait that influence fitness in both the source and sink habitats, 4) speciation under conditions of a soft-sink habitat, and 5) eco-evolutionary responses to therapy that target the source habitat or the predominant cancer cell type. Results from goals 1–4 will be familiar to those familiar with the expansive literature on source sink dynamics (Diffendorfer, 1998). We demonstrate how source-sink dynamics are applicable to cancers and can produce the observed spatial variations in genetic and phenotypic properties of cancer cells, and suggest critical issues in designing patient treatment strategies.

## MATERIALS AND METHODS

Here, we model a source habitat and consider three variations of an adjacent sink habitat: a black-hole sink, a hard sink, and a soft sink (Gravel et al., 2010; Borovski et al., 2011; Gerlinger et al., 2012). In all cases the source habitat will generally be A and the sink B. When habitat B is a black hole sink, any cancer cell that migrates from A to B dies instantly. When habitat B is a hard sink, cancer cells cannot proliferate but they may die off slowly at some fixed per capita rate. When habitat B is a soft sink, it can sustain a smaller population of cancer cells than habitat A and becomes a sink habitat only because of the disproportionate number of migrants into B than out of B.

Within habitat A and within habitat B when it is a soft sink, cancer cells directly compete for space and limited resources. While these cancer cells do not directly compete with cells in the other habitat, they interact indirectly due to the dispersal of cancer cells between the source and sink regions. This dispersal is represented by a per capita migration rate that describes the probability that an individual in one habitat migrates to the other. This migration rate can also represent the habitat shifting underneath stationary cancer cells, such as when vasculature becomes unstable, shifting the boundary between well perfused and poorly perfused microenvironments.

The competition of cancer cells within a habitat and migration between habitats is analyzed using a game theoretic approach in which a *G function* couples ecological (population) and evolutionary (strategy) dynamics (Vincent and Brown, 2005). This framework is built upon the three principles of Darwin's theory of natural selection: there must be heritable variation,

there must be a "struggle for existence" (i.e., limited resources and space prevent all populations from growing exponentially), and heritable variation must influence this struggle. In the G function approach, one considers a focal (or virtual) cell with strategy (= heritable phenotype), $v$, which, along with the strategies (u) and densities ($N_A, N_B$) of competing cancer cell populations, determines the cell's expected fitness or proliferation rate. For example, $u$ may represent expression levels of key proteins implicated in cellular proliferation. Here, we let $u = (u_1, ...u_n)$ be the vector of strategies of the $n$ different species where $u_i$ represents the strategy of species $i = 1, ..., n$. Note that the length of this vector can change dynamically in time: as species diversify or go extinct, the vector will correspondingly expand or shrink. Here, we assume that species are identical except for the values of their strategies. Let $N_A = \left(N_A^1, ..., N_A^n\right)$ and $N_B = \left(N_B^1, ..., N_B^n\right)$ be the vector of population sizes in the source habitat $A$ and the sink habitat $B$, where $N_A^i$ represents the population size of species $i$ in habitat $A$. Let $F_A$ and $F_B$ describe the fitness of a cancer cell in the source ($A$) and sink habitats ($B$), respectively. We assume that fitness within a habitat is only influenced by the cells within that habitat where $F_A\,(v,\ u,\ N_A)$ and $F_B\,(v,\ u,\ N_B)$.

We assume random migration between the two habitats where $m_A$ is the per capita migration rate of cells from habitat $A$ to habitat $B$, and vice versa for $m_B$. We assume these migration rates are constant but this could be relaxed to include density-dependent habitat selection (Rosenzweig, 1981; Tarjuelo et al., 2017) and migration rates themselves could become a component of the heritable strategy (Morris, 1991; Schmidt et al., 2000). The number of cells in the source increases as the source cells proliferate, and through incoming migration from the sink. The number of cells in the source decreases due to outgoing migration to the sink. These dynamics also apply, respectively to the sink. The change in population size of each habitat can be written as:

$$\frac{dN_A^i}{dt} = N_A^i F_A\,(v,\ u,\ N_A)|_{v=u_i} - m_A N_A^i + m_B N_B^i$$

$$\frac{dN_B^i}{dt} = N_B^i F_B\,(v,\ u,\ N_B)|_{v=u_i} - m_B N_B^i + m_A N_A^i$$

To simulate the eco-evolutionary dynamics of cancer cells, we treat our habitats as different states in the life history of cancer cells, coupled via migration. This framework allows us to capture the population dynamics of cancer cells with a *population projection matrix*. Each entry in the matrix represents transitions between the two life history states. An ecologically inclined reader may notice that this is analogous to the Leslie matrix for structured populations. Our population projection matrix, denoted by P, can be written as:

$$P = \begin{bmatrix} F_A - m_A & m_B \\ m_A & F_B - m_B \end{bmatrix}$$

Then, we can represent our population dynamics as

$$\begin{pmatrix} \frac{dN_A^i}{dt} \\ \frac{dN_B^i}{dt} \end{pmatrix} = P \begin{pmatrix} N_A^i \\ N_B^i \end{pmatrix}$$

Though we can use this matrix to simulate population dynamics, we must still construct a fitness function to capture strategy dynamics. We can define this fitness function as the dominant eigenvalue of the population projection matrix since this is what controls (approximates) long-term behavior (Vincent et al., 1993; Vincent and Brown, 2002). In other words, we have

$$G\,(v, u, N_A,\ N_B) = max(Re(\lambda_i))$$

where $\lambda_i$ are the eigenvalues of P. Then, the evolutionary dynamics of $u_i$ depends on the local gradient of the $G$ function: how the fitness of the cells change due to perturbations in the trait value and the rate at which cells can climb this fitness gradient. Mathematically, the evolutionary dynamics of species i can be formalized (Vincent et al., 1993) as:

$$\frac{\partial u_i}{\partial t} = c * \left.\frac{\partial G}{\partial u}\right|_{v=u_i}$$

where $c$ is a measure of additive genetic variance, in accordance with Fisher's fundamental theorem of natural selection. The $\frac{\partial G}{\partial u}$ term captures the local gradient of the fitness generating function at $v = u_i$. To reiterate, a cell's fitness, $G\,(v,\ u,\ N_A,\ N_B)$, depends on its own strategy, $v$, the strategies of the other tumor cells, $u$, and the population sizes of tumor cells in the source habitat ($A$) and the sink habitat ($B$), $N_A$ and $N_B$. The fitness generating function, $G$, describes the ecological (changes in total and local population size, $N_A,\ N_B$), and $\frac{\partial G}{\partial u}$ describes the evolutionary dynamics (changes in the populations heritable strategy values, $u$). If at $v = u_i$, $G\,(v,\ u,\ N_A,\ N_B) = 0$ then $N_A^i$ and $N_B^i$, the total population size of species $i$, will increase and vice-versa for $G < 0$. The direction of the strategy dynamics can be seen by the adaptive landscape which plots $G$ versus the focal cell's strategy, $v$, while holding the other cells' strategies (u) and population densities ($N_A, N_B$) constant. A species strategy $u_i$ will climb the adaptive landscape until the system reaches a stable point where it is both evolutionarily ($\partial G/\partial u|_{v=u_i} = 0$) and ecologically stable ($\partial N_A^i/\partial t = \partial N_B^i/\partial t = 0$). As $u,\ N_A,\ N_B$ change, so too does the entire adaptive landscape, sometimes dramatically (Vincent and Brown, 2005).

We now consider the eco-evolutionary outcomes when the system starts with just a single species: $n = 1$. Interestingly, the eco-evolutionary stable point can occur at either a minimum or a maximum of the adaptive landscape (Cohen and Brown, 1999). If the stable point is at a maximum of the landscape where ($\partial^2 G/\partial u^2 < 0$), the cancer cells have evolved to their evolutionary stable strategy (ESS) (Vincent and Brown, 1988). On this other hand, if the stable point is at a minimum of the landscape ($\partial^2 G/\partial u^2 > 0$), the cancer cells might speciate (= evolutionary branching, Geritz et al., 1998), creating two distinct cancer cell types or "species" each with its own unique strategy $u_1$ and $u_2$. These species will climb to their respective peaks of the adaptive landscape to reach their own unique ESS. Hence, when there is just one species: $u = u_1$ and $N_A = N_A^1,\ N_B = N_A^1$. When there are more than one species $u,\ N_A,\ N_B$ becomes vector valued. Each species will have its own strategy and its own population distribution between the source and sink habitats.

The habitat-specific population dynamics $\frac{dN_A}{dt}$ and $\frac{dN_B}{dt}$, and habitat-specific fitnesses $F_A$ and $F_B$, for the black-hole sink, hard sink, and soft sink are described in **Box 1**. We set $r_A = r_B = 0.025$ (many patient's tumors experience growth at rates of 2.5 % per day) as the maximum growth rate of each habitat. The functional forms for carrying capacities and death rates are provided in **Box 1**. The strategy of the focal cell, $v$, influences either its habitat-specific carrying capacity or habitat-specific death rate: $K_A(v)$, $K_B(v)$, and $d_B(v)$. For the relationships between $K_A(v)$ and $K_B(v)$ and $v$, we use a quadratic equation. The parabolas were scaled so that at $v = 1$, $K_A(1) = 100$ (maximum achievable carrying capacity in habitat A) and $K_B(1) = 25$ (the less well perfused habitat can support just 1/4 as many cells when the cells are best adapted for A). At $v = 0$ (best adapted for habitat B), we let both habitats have the same carrying capacity of 50: $K_A(0) = K_B(0) = 50$. Thus, as $v$ goes from 0 to 1 the cancer cell's carrying capacity in habitat A goes from 50 to 100, and its carrying capacity in habitat B declines from 50 to 25 (**Figure 2**).

To determine the effects of migration on the dynamics of the evolutionary game, we numerically solved for the ESS. We consider values for $m_A$ and $m_B$ in the range from very slow migration ($m_A = m_B = 10^{-4}$) to very fast migration ($m_A = m_B = 10^0$). We initialize each numerical run of the model by assuming that the cancer cells originate primarily in the source habitat A. In this way, we set the population density in habitat $A$ to relatively full, $N_A(0) = 95$, habitat $B$ to relatively empty $N_B(0) = 5$, and all cells with the strategy that maximizes fitness in habitat $A$, $v = 1$ at time zero.

## Modeling Treatment

The models for the black-hole, hard and soft sinks in **Box 1** determine the cancer's ecological and evolutionary dynamics in the absence of patient treatment. Within the context of the soft-sink model, we consider two types of treatment, habitat dependent and cancer cell phenotype dependent. Habitat dependent treatments are more effective in the source habitat than the sink habitat and have been previously modeled (Fu et al., 2015; Moreno-Gamez et al., 2015). In cancer, chemotherapeutic drugs perfuse more thoroughly through regions near the vasculature (source habitat) than habitats farther from vasculature (sink habitat). The diffusion dynamics that reduce nutrient concentrations away form blood vessels also reduces the concentration of systemically delivered drugs (Perez-Velazquez and Rejniak, 2020).

We additionally present a model for phenotype dependent treatment, where drug efficacy depends on the strategy of the cancer cells. This represents a targeted therapy that is maximally effective for a given strategy value and drug efficacy then declines as the cancer cells' strategy deviates from the therapeutically optimal value.

To consider a habitat-dependent treatment, we add a death term that represents a habitat-specific therapy-induced death rate:

$$\frac{dN_A^i}{dt} = N_A^i F_A - m_A N_A^i + m_B N_B^i - \gamma_A N_A^i$$

$$\frac{dN_B^i}{dt} = N_B^i F_B - m_B N_B^i + m_A N_A^i - \gamma_B N_B^i$$

where $\gamma_x$ represents the fraction of cells that die due to treatment in habitats A and B. We set $\gamma_A = 0.05$ and $\gamma_B = 0$ due to the increased delivery of drug to the well vascularized source habitat.

We model strategy dependent treatment as:

$$\frac{dN_A^i}{dt} = N_A^i F_A - m_A N_A^i + m_B N_B^i - \gamma\,(v) N_A^i$$

$$\frac{dN_B^i}{dt} = N_B^i F_B - m_B N_B^i + m_A N_A^i - \gamma\,(v) N_B^i$$

where $\gamma\ (u)$ captures how effective the treatment is as a function of the cancer cell strategy, $v$. Specifically, we use the following form for $\gamma\ (v)$ :

$$\gamma\ (v) = \gamma_M exp(-\frac{(v - v_{opt})^2}{\sigma_t})$$

where $\gamma_M$ represents maximal drug efficacy set here to 0.05, $v_{opt}$ is the cancer cell strategy at which the drug is maximally effective ($v = 1$), and $\sigma_t$ is a measure of how "general" the treatment is set here to 0.05. As the cancer cell's strategy deviates from $v_{opt}$, drug efficacy declines according to a Gaussian curve. **Figure 3** depicts the shape of this functional form.

**BOX 1 |** Mathematical model of all three sink habitat scenarios including the population dynamics, habitat fitness functions, and the properties of the habitats with respect to a cell's strategy.

| | **Black-Hole Sink** | **Hard Sink** | **Soft Sink** |
|---|---|---|---|
| Population Dynamics | $\frac{dN_A^i}{dt} = N_A^i F_A - m_A N_A^i$ | $\frac{dN_A^i}{dt} = N_A^i F_A - m_A N_A^i + m_B N_B^i$<br>$\frac{dN_B^i}{dt} = N_B^i F_B - m_B N_B^i + m_A N_A^i$ | $\frac{dN_A^i}{dt} = N_A^i F_A - m_A N_A^i + m_B N_B^i$<br>$\frac{dN_B^i}{dt} = N_B^i F_B - m_B N_B^i + m_A N_A^i$ |
| Habitat fitness | $F_A = r_A \left(\frac{K_A(v) - N_A}{K_A(v)}\right)$ | $F_A = r_A \left(\frac{K_A(v) - \sum_{j=1}^{n} N_A^j}{K_A(v)}\right) F_B = -d_B\,(v)$ | $F_A = r_A \left(\frac{K_A(v) - \sum_{j=1}^{n} N_A^j}{K_A(v)}\right)$<br>$F_B = r_B \left(\frac{K_B(v) - \sum_{j=1}^{n} N_B^j}{K_B(v)}\right)$ |
| Habitat properties | $K_A\,(v) = a_{KA} \times (v - h_{KA})^2 + k_{K_A}$<br>$a_{K_A} = -50$<br>$h_{K_A} = 100$<br>$k_{K_A} = 1$ | $K_A\,(v) = a_{KA} \times (v - h_{KA})^2 + k_{K_A}$<br>$K_B\,(v) = a_{d_B} \times (v - h_{d_B})^2 + k_{d_B}$<br>$a_{K_A} = -50\ a_{d_B} = 0.01$<br>$h_{k_A} = -50\ h_{d_B} = 0$<br>$k_{K_A} = 100\ k_{d_B} = 0.005$ | $K_A\,(v) = a_{KA} \times (v - h_{KA})^2 + k_{k_A}$<br>$K_B\,(v) = a_{k_B} \times (v - h_{k_B})^2 + k_{k_B}$<br>$a_{K_A} = -50\ a_{k_B} = -25$<br>$h_{k_A} = -50\ h_{k_B} = 0$<br>$k_{K_A} = 100\ k_{k_B} = 50$ |

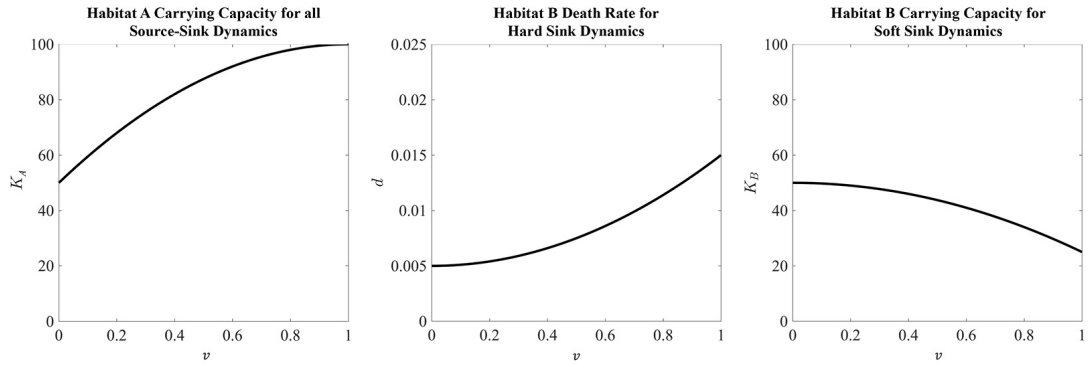


**FIGURE 2 |** Habitat properties for the source and sink habitats defined in **Box 1**. The source habitat, which is the same for all three sink habitat analyses, has a maximum carrying capacity at a strategy equal to 1. This carrying capacity then falls as the strategy moves away from strategy value 1 toward a minimum carrying capacity of 50 with a strategy equal to 0. For the hard sink dynamics, habitat B is defined by a death rate. Here the death rate is minimized at a strategy equal to 0. This death rate increases at the strategy moves away from 0 to a maximum death rate of 0.015 at a strategy equal to 1. Lastly, the carrying capacity of the soft sink habitat B has a maximum of 50 at a strategy equal to 0. As the strategy moves toward 1, the carrying capacity falls to a value of 25.

# RESULTS

## Black-Hole Sink

The black hole sink supports no population. From the perspective of the source population, migration to the black-hole sink represents a per capita death rate, shown in **Figure 4**. From the perspective of the cancer patient, any movement of cancer cells into surrounding tissue or extravasation of CTCs into completely inhospitable tissues is beneficial.

The ESS for all values of $m_A$ is $u^* = 1$, as there is no tradeoff for balancing fitness in the source versus the sink habitat. With very slow migration rates, $m_A = 10^{-4}$, the source habitat can maintain a population density very close to its carrying capacity. As the migration rate increases, the ESS population size falls until a critical value of $m_A = r_A = 0.025$. When the migration rate is greater $r_A$, the sink habitat will drain the source population to extinction.

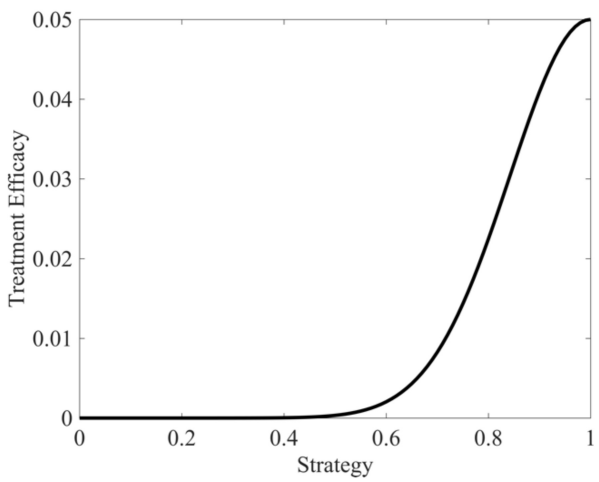


**FIGURE 3 |** Targeted therapy efficacy as a function of trait value. Therapeutic efficacy is maximized when $v = 1$ and drops off in a Gaussian fashion as trait values diverge from $v = 1$.

## Hard Sink

In a hard sink, the ESS is significantly altered by the migration rates. Due to cells' exposure to the sink habitat $B$ where the strategy that maximizes fitness is $u^* = 0$, the ESS $u^*$ is not always equal to 1 (**Figure 5**). In general, when $m_A$ is very low, regardless of the migration rate $m_B$, the ESS is $u^* = 1$, as cancer cells mostly reside in and experience habitat $A$. When the migration back to the source, $m_B$, is negligible, we can again see the critical $m_A = r_A = 0.025$ where, the source habitat drains the source habitat to extinction.

In the absence of migration from the sink habitat to the source, the hard sink acts like the black hole sink with the exception that during the transient dynamic to extinction, there can still be a sizable population in the sink habitat. Such transients are difficult to detect from histologies of biopsy samples, though indirectly one may be able to estimate birth and death rates of cancer cells from immunohistochemical stains such as Ki67 (a proliferation marker) and CC3 (an apoptosis marker of cell death) (Johnson et al., 2019).

When there is migration from the sink back to the source, then the eco-evolutionary prospects of the source habitat and tumor change dramatically. This becomes of interest as cancer cell movement from necrotic regions (micro-scale or large scale) or regions of hypoxia is likely within tumor microenvironments, especially when cancer cells remain relatively stationary while the habitats themselves form and shift in space.

Of most interest is when the migration rates $m_A$ and $m_B$ are such that the source population supports a population in the sink habitat. Under these migration rates, the ESS is a compromise between $u^* = 0$ and $u^* = 1$. A generalist species evolves that sacrifices carrying capacity in the source habitat for survivorship in the sink. In this way, the presence of a hard sink habitat pulls the ESS of the entire population, including those cells in the source habitat, away from the optimal strategy of the

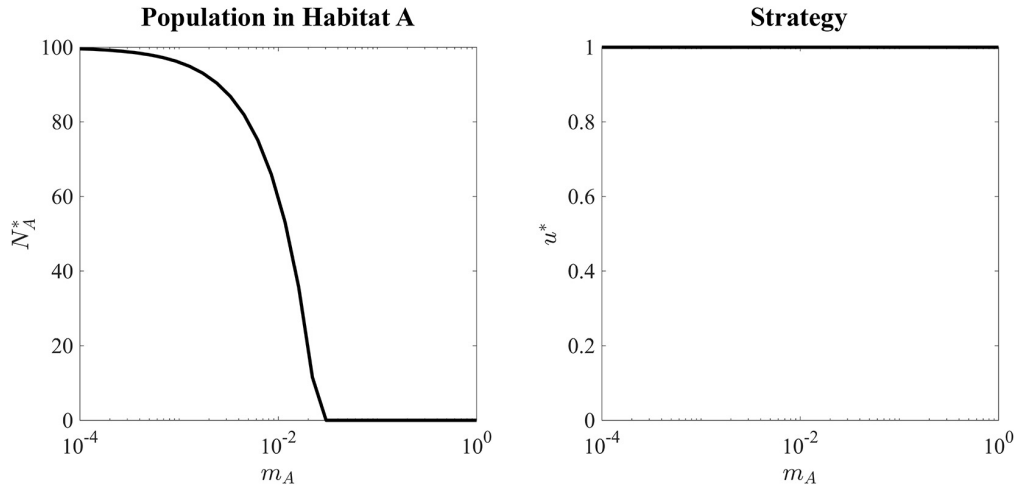


**FIGURE 4 |** Ecological and evolutionary ESS of a black-hole sink. Left panel shows the ESS population density in the source habitat as a function of migration rate. As migration rate increases, more cells are constantly migrating out of Habitat A resulting in a lower sustainable population. Right panel shows that for every value of migration rate, the ESS value is equal to 1, as this strategy maximizes the carrying capacity in the source habitat.

source habitat. Survival and reseeding from the sink becomes ecologically and evolutionarily consequential.

For example, **Figure 6** shows how the adaptive landscape, cancer strategy value, and habitat-specific population sizes change over time for $m_A = m_B = 10^{-1}$. When the source habitat starts out relatively full and the sink habitat relatively empty, all cells have a strategy maximizing fitness in the source habitat, the adaptive landscape shows that decreasing the strategy value will increase overall fitness, $G$. The cancer cells' strategy climbs the adaptive landscape until the slope of the landscape is zero ($\partial G/\partial u = 0$) and the population sizes equilibrates so that fitness is 0 ($G = 0$). The ESS at this stable point is $u^* = 0.5358$, and the distribution of individuals between the two habitats is $N_A^* = 63.18$ and $N_B^* = 58.57$.

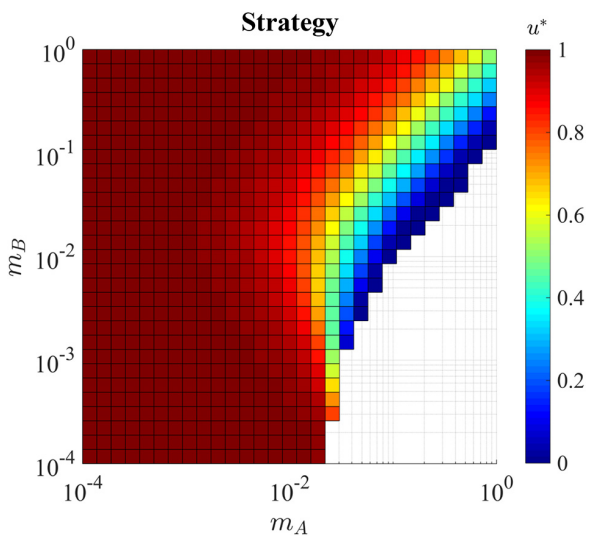


**FIGURE 5 |** Evolutionary stable strategy in the presence of a hard sink for all combinations of $m_A$ and $m_B$. With both migration rates slow (bottom left corner) the ESS remains equal to one as the exposure to the sink habitat is minimal. This is the same for the upper left corner where cells migrate at a slow rate out of habitat A and quickly migrate back, again minimizing the exposure to the sink habitat. In the lower right, the population can not survive as cell migrate quickly out of the source habitat and get 'stuck' in the hard sink, eventually dying before being able to return to the source where proliferation is possible. In the upper right corner where cells are migrating back and forth between the habitats quickly, the exposure to the sink habitat pulls the ESS u* away from 1 and toward the ESS of the sink habitat which is 0.

This generalist strategy of $u^* = 0.5358$ allows for a total population size of $N^* = 121.75$ that is greater than the maximum population that can be sustained by the source habitat alone, 100. For all combinations of $m_A \approx m_B$ where both are greater than $\approx 10^{-2}$, the total ESS population size is greater than 100, reaching a maximum possible N* of 124.1 (**Figure 7**). With a sufficiently low $d_B$ (v), the sink habitat can even harbor more individuals than the source habitat. For these reasons, the sink habitat can influence the ESS by selecting for a population wide $u^* < 1$. This evolution allows the sink habitat to become a large reservoir of cells that can repopulate the source habitat following perturbations such as therapy. Population size alone cannot be used to infer which microhabitats in tumors are sources and hard sinks.

## Soft Sink

In a soft sink, both habitats can support a population independently, allowing for positive per capita growth rates in each habitat when population sizes are small. This creates an opportunity not available in black hole or hard sinks for the ESS to contain two species when migration rates are relatively low. For example, **Figure 8** shows the adaptive landscape and evolutionary dynamics over time for $m_A = m_B = 10^{-3}$. Under the initial conditions, where habitat $A$ is relatively full, habitat $B$ relatively empty, and all cells have a strategy maximizing fitness

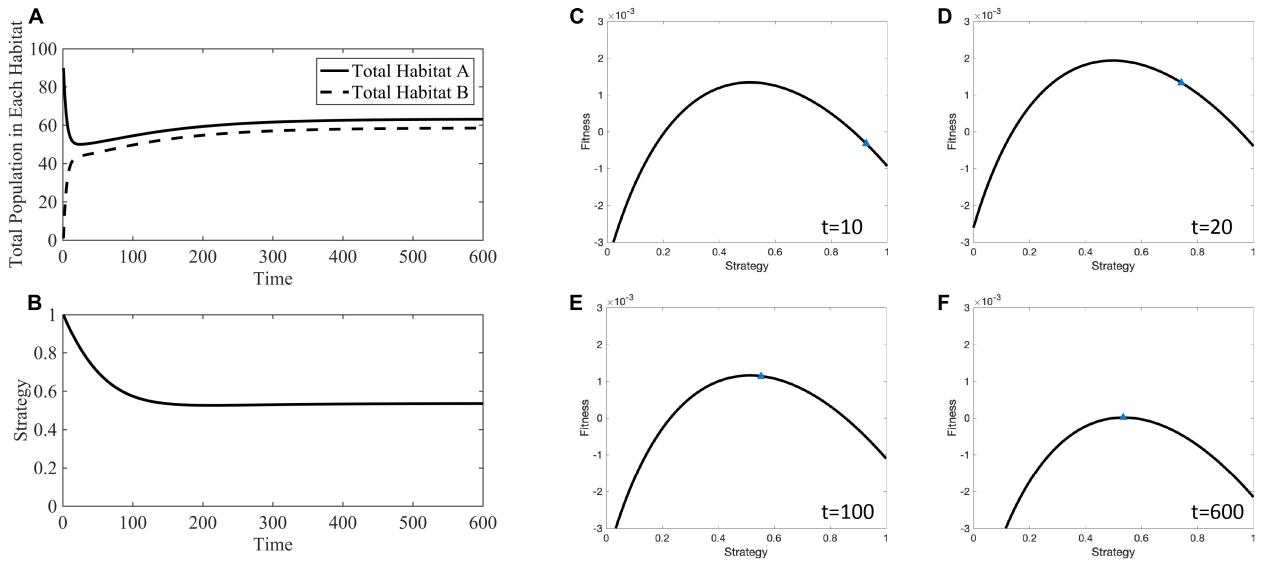


**FIGURE 6 |** Ecological and evolutionary dynamics in the presence of hard sink where $m_A = m_B = 10^{-1}$. **(A)** Population dynamics of habitat A and B showing the quick migration out of the initially large source population to fill the sink habitat followed by dynamics toward the stable population densities. **(B)** The strategy value over time decreases away from 1 to a generalist strategy between 0 and 1. **(C)** The adaptive landscape at the initial conditions, where the source habitat is relatively full, the sink habitat is relatively empty, and all cells have a strategy maximizing fitness in the source habitat shows that fitness could be greatly increased by decreasing the strategy value and climbing the adaptive landscape. **(D–F)** As time progresses the strategy value climbs to an ESS of $u^* = 0.5358$. **Supplementary Movie 1** provides a movie of the panels presented in **(C–F)**.

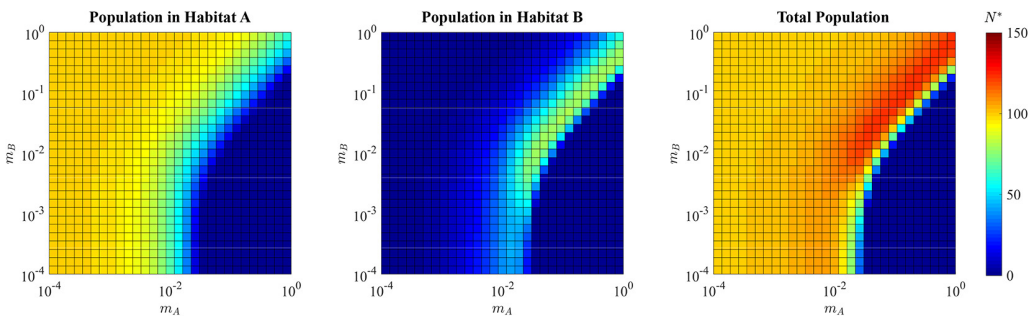


**FIGURE 7 |** ESS population density of habitat A, habitat B, and the total combined population in the presence of a hard sink. The population is specialized to habitat A when the migration rate $m_A$ is relatively low (lower left quadrant and upper left quadrant of each panel) reaching the carrying capacity of habitat A with relatively low density in habitat B. When the migration out of the source habitat A is fast and there is low migration back from the sink (lower right quadrant), the population declines to extinction. When there is a balance of migration rates (upper right quadrant), a generalist strategy allows for cells in both habitat A and habitat B, resulting in a total ESS population greater than would be available with only the source sink.

in habitat $A$, the adaptive landscape shows that decreasing the strategy value will increase fitness. Interestingly, the convergent stable point is at a minimum of the landscape ($\partial^2 G/\partial u^2 > 0$), and the cancer cells should speciate, creating two distinct populations or "species" each with its own unique strategy. These species climb their respective peaks of the adaptive landscape to reach an ESS with two species.

Each species becomes a specialist on their respective habitat. In this way, species 1 has a strategy of $u^* \approx 1$ that maximizes carrying capacity in habitat $A$, while species 2 has a strategy of $u^* \approx 0$ that maximizes carrying capacity in habitat $B$ (bottom left corner of **Figure 9**). In cancer, this likely explains some of the heterogeneity in cancer cell types (Lloyd et al., 2016), and is most likely to promote diversity when habitats are relatively large and contiguous, thus reducing migration rates between them. In line with this, secondary tumors in different tissue types from the primary tumor will evolve cancer cells with quite distinctive phenotypes appropriate to the specific tissue type (Klein et al., 2002; Quinn et al., 2021). Such divergences have also been seen in 3-D cancer cell culture experiments (Ruud et al., 2020).

When migration rates are relatively high for both $m_A$ and $m_B$, the rapid movement of cells between habitats selects for a single generalist species (upper right corner of **Figure 9**). Interestingly, when $m_A$ and $m_B$ are at opposite extremes (consider the upper left corners and lower right corners of **Figure 9**) the ESS tends to specialize on the habitat with high immigration and

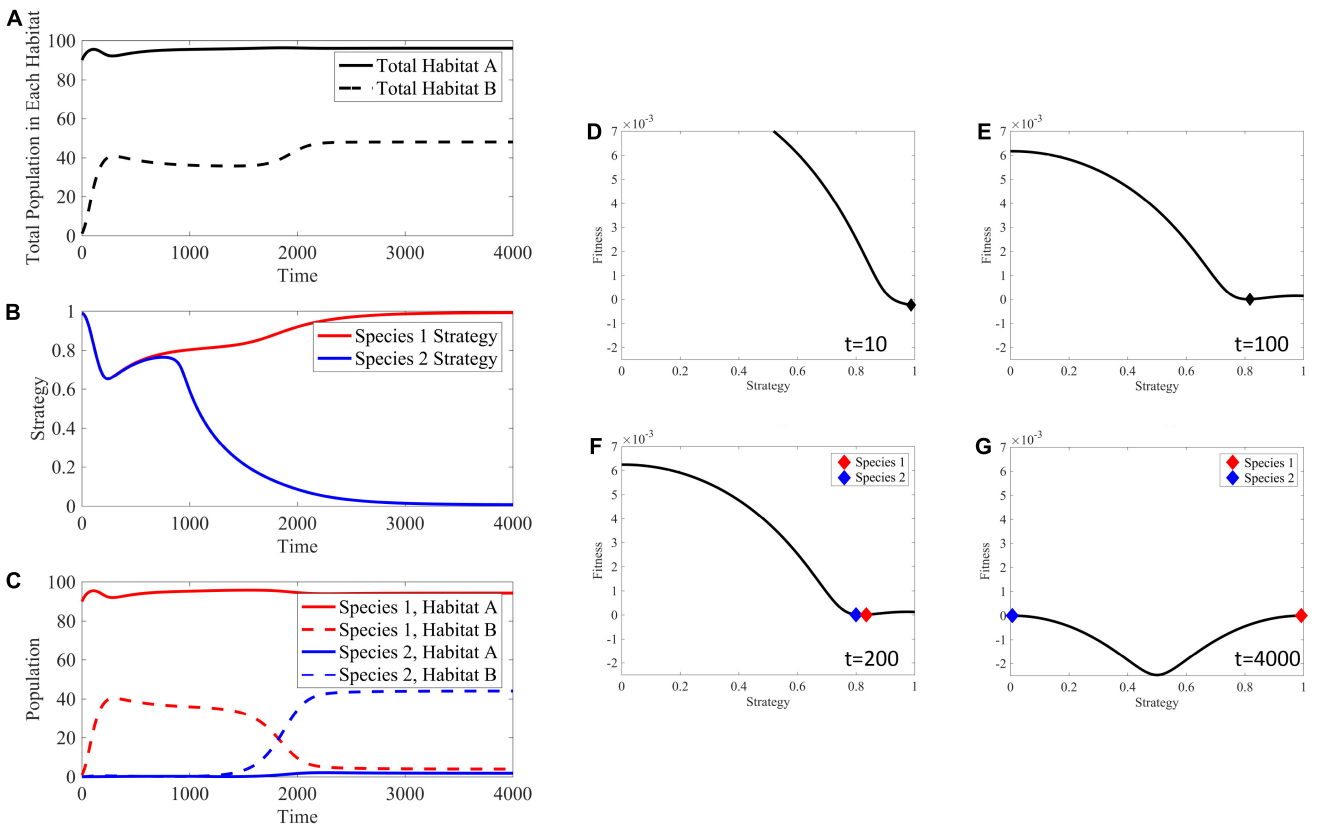


**FIGURE 8 |** Ecological and evolutionary dynamics in the presence of soft sink where $m_A = m_B = 10^{-3}$. **(A)** Population dynamics of habitat A and B showing habitat B filling up with migrant cells from the source habitat A, then both cell populations maximizing their respective carrying capacities after speciation. **(B)** The strategy value over time, showing a speciation event at around time 1000. These dynamics are seen in the panels **(D–G)** with respect to the underlying adaptive landscape. **(C)** The population densities broken down between both species and habitat. Species 1 is the only species filling both habitats before the speciation event. Afterwords, habitat B is filled with species 2 having a strategy equal to 0 maximizing fitness in this habitat, while habitat A remains filled with species 1. **(D)** The adaptive landscape at the initial conditions, where the source habitat is relatively full, the sink habitat is relatively empty, and all cells have a strategy maximizing fitness in the source habitat shows that fitness could be greatly increased by decreasing the strategy value and climbing the adaptive landscape. **(E)** The population evolves to a minimum in the adaptive landscape. **(F,G)** Speciation occurs and the individual species climb their respective peaks. **Supplementary Movie 2** provides a movie of the panels presented in **(D–G)**.

low emigration. For example, the upper left corner has high immigration into habitat $A$ from habitat $B$, and low emigration from habitat $A$ to habitat $B$. Here we see the ESS selects for $u^* = 1$, which is the optimal strategy for habitat $A$. The same is true, but opposite for the lower right corner.

The migration rates favoring a single generalist, single specialist, and speciation to two coexisting specialist species are shown in **Figure 10**.

For the adaptive landscape example shown in **Figure 8** where $m_A = m_B = 10^{-3}$, the total ESS population is $N^* = 144.2$, well above the carrying capacity of the source habitat alone (**Figure 11**). In the single specialist regions, the total population is near or a little above the carrying capacity of the habitat to which the species is specialized. The region where a single generalist strategy is the ESS, like that in the hard sink, can also sustain total populations greater than each of the habitats individually.

It is important to note that if each habitat can support individuals alone, the definition of the source and sink habitat are context dependent. There are indeed regions where the migration rates and populations in each habitat make habitat $B$ the source where $F_B > 0$ and habitat $A$ the sink where $F_A < 0$.

## Consequences of Habitat-Dependent and Phenotype-Dependent Therapies

Witihin the context of a soft-sink, we set migration values to $m_A = m_B = 10^{-3}$ so as to be in the speciation regime of the phase portrait, and analyze the eco-evolutionary dynamics of cancer cells under two types of therapy: habitat dependent and phenotype dependent. First, we consider habitat treatment under which all species in habitat A (the source habitat) are subject to the effects of therapy, regardless of their strategy. Species in habitat B are not directly affected by this treatment. In ecology, this is analogous to the application of pesticide to a portion of farmland. In cancer, it pertains to the pharmacokinetics of drug delivery through vasculature and the size of the tumor. The drug may only reach certain areas of the tumor at high concentrations (source habitats) but is unable to permeate other

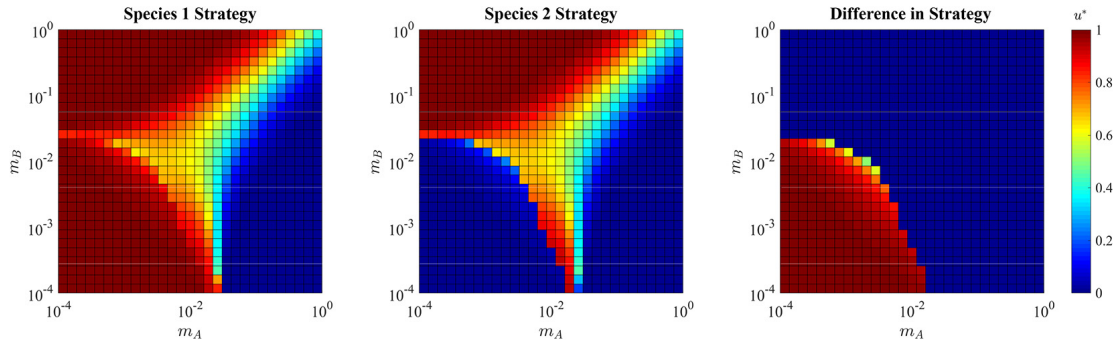


**FIGURE 9 |** ESS in the presence of a soft sink. Due to the possibility of speciation, the strategy for both species 1 and species 2 are shown independently in the first two panels, and the differences between them is shown in the right panel. Where the population is specialized to habitat A (upper left quadrant) the single species ESS converges on $u^* = 1$. Where the population is specialized to habitat B (lower right quadrant) the single species ESS converges on $u^* = 0$. Where the population takes a single generalist approach (upper right quadrant) the ESS compromises and converges to a strategy between 0 and 1. Where the population speciates (lower left quadrant), each species converges to strategy that maximizes fitness in their respective habitats: $u^* = 0$ and $u^* = 1$.

regions of the tumor (sink habitats), sheltering these cells from the effects of therapy.

To simulate habitat treatment, we simply eliminate 5% of the cells in habitat A at each time step in the simulation. This changes the source habitat into a sink habitat and vice versa.

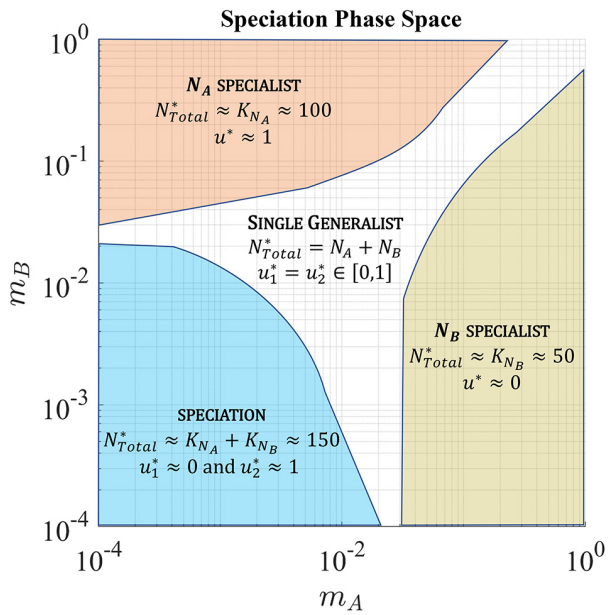


**FIGURE 10 |** In a soft sink, migration rates, $m_A$ and $m_B$, determine whether the ESS has a single generalist species, single specialist species, or speciation resulting in the coexistence of two specialist species. The population is specialized to habitat A (upper left quadrant) when the rate of cell migration out of habitat A is low and the rate of cell migration back into habitat A is high. Alternatively, the population is specialized to habitat B (lower right quadrant) when the rate of cell migration out of habitat B is low and the rate of cell migration back into habitat B is high. When the migration between both habitats in low, the population speciates with each species specializing on one habitat. In the other regions, the ESS converges to a single generalist species with a strategy that represents a compromise between the two habitats.

As such, cells in habitat A go extinct, while the cell populations in habitat B remain at their carrying capacity (**Figures 12A,C**). Since habitat A can no longer support any cells, species 1, which formerly specialized in habitat A, evolves toward a strategy of $v = 0$ converging on that of species 2 (**Figure 12B**). Even as species 1 evolves toward specializing on habitat B its population declines, potentially to extinction, as a consequence of species 2 already being a habitat B specialist. The ESS goes from two specialist species prior to therapy to a single specialist species following therapy. These changes can clearly be seen in the adaptive landscapes (**Figures 12D–G**). Before the application of therapy (**Figure 12D**), there exist two peaks on the adaptive landscape: one at $v = 1$, habitat A which species 1 occupies, and one at $v = 0$, habitat B which species 2 occupies. However, once therapy is administered, the two peaks of the adaptive landscape change into a single peak at $v = 0$, corresponding to being a habitat B specialist. Species 1 is initially entirely unfit for this habitat (**Figure 12E**), but eventually evolves (**Figure 12F**) and converges on the strategy at $v = 0$ (**Figure 12G**).

Now, consider a phenotype-dependent therapy or targeted therapy whose efficacy depends on the species' trait value, regardless of their habitat. In ecology, the targeted therapy may be analogous to fish harvesting by humans, with the species' trait representing fish body size. In cancer, the targeted therapy (Herceptin) may target a specific protein (HER-2) in a cancer metabolic pathway. In both instances, one can imagine that the targeted therapy will not be effective at low values of the trait (a small fish or a cancer cell with a low expression of the target protein) but may be highly effective for high values of the trait.

We simulate targeted therapy by using a maximal efficacy of 5% at the cancer cell strategy at which the drug is maximally effective ($v = 1$), with efficacy falling as $v$ diverges from 1 (**Figure 3**). First, consider the overall dynamics in **Figure 13A**. We note that the total population (combined over both species) in habitat B remains remarkably constant for the entirety of the simulation. Because most of the individuals in habitat B have a strategy value less effected by the targeted therapy, cancer cells in habitat B suffer a smaller decline from therapy than those in

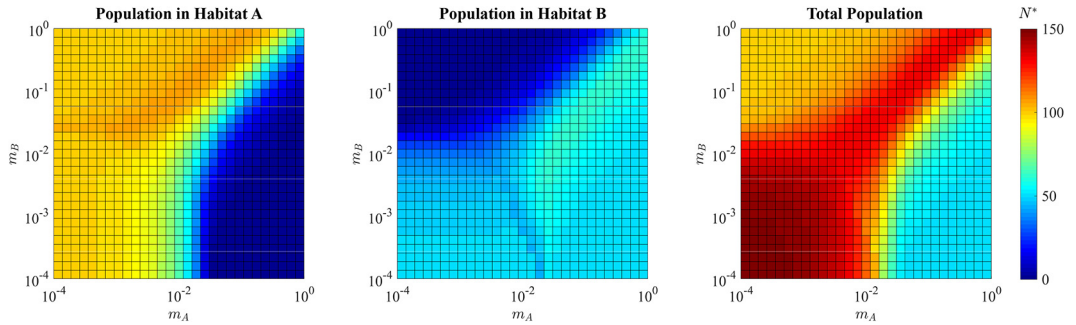


**FIGURE 11 |** ESS population density of habitat A, habitat B, and the total combined population in the presence of a soft sink. Where the population is specialized to habitat A (upper left quadrant) the ESS population density reaches the carrying capacity of habitat A, with relatively low density in habitat B. Where the population is specialized to habitat B (lower right quadrant) the ESS population density reaches the carrying capacity of habitat B, with relatively low density in habitat A. Where the population evolves a generalist strategy (upper right quadrant) the ESS population can exceed the maximum carrying capacity provided in the source habitat, with substantial numbers of cancer cells in both habitat A and habitat B. Where the population speciates (lower left quadrant), each species can nearly reach its maximal carrying capacity within its preferred habitat, allowing the total ESS population to approach $N^* = 150$.

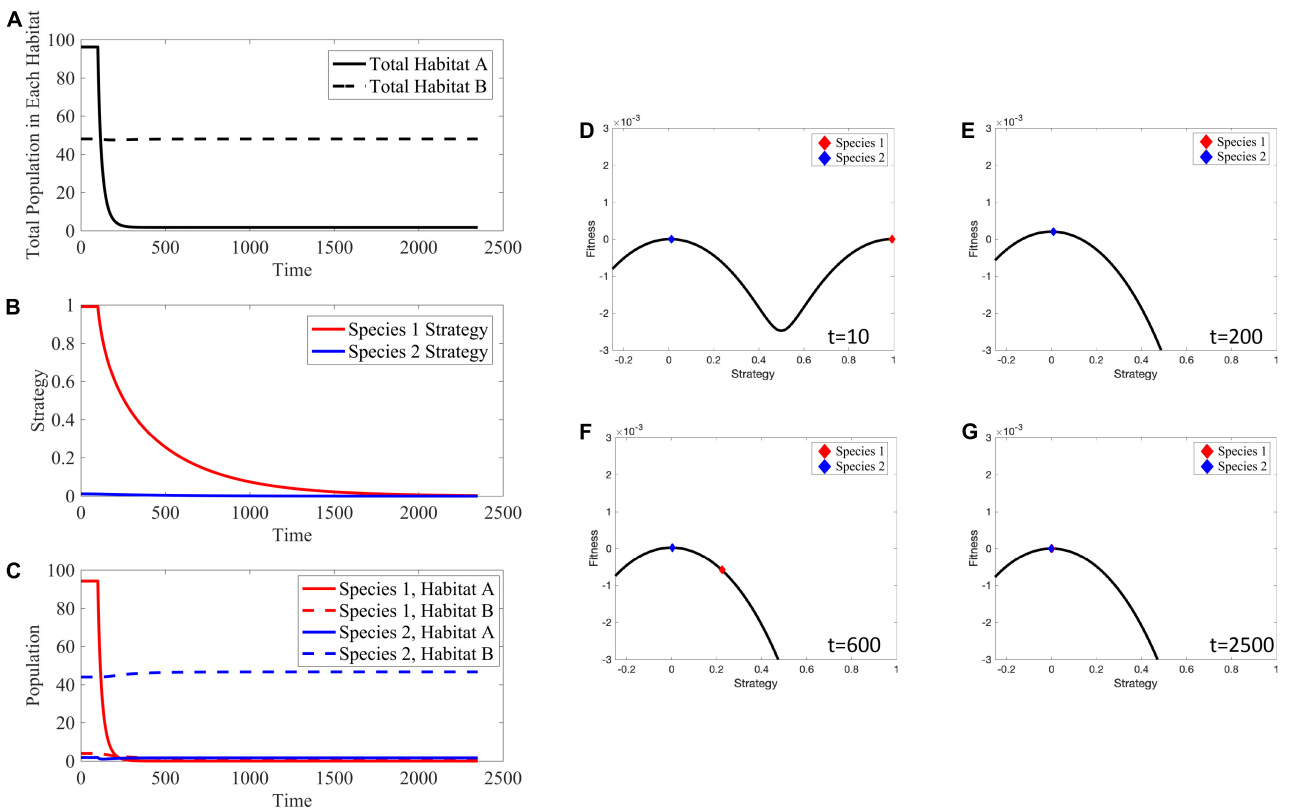


**FIGURE 12 |** Effects of Habitat Treatment. Habitat A can no longer support any cells, and evolution drives species 1 to evolve toward $v = 0$ to persist in habitat B. **(A)** The total population in habitat A crashes to 0, while the total population in habitat B remains at its carrying capacity. **(B)** Since habitat A is no longer viable, species 1 evolves its strategy toward $v = 0$ in an attempt to remain extant in habitat B. **(C)** Species 1 crashes in habitat A after the application of treatment and is not able to evolve its strategy fast enough to persist in habitat B. There is little to no change in population density of species 2 in habitat B. **(D–G)** Depictions of the adaptive landscape over time. Before application of therapy, there are two peaks in the adaptive landscape, corresponding to the two viable habitats. After therapy is administered, the peak corresponding to habitat A vanishes and species 1 evolves toward the peak at $v = 0$. **Supplementary Movie 3** provides a movie of the panels presented in **(D–G)**.

habitat A. Because the targed therapy is most effective against cancer cells specialized on habitat A, the population in A drops dramatically immediately after therapy is administered. At least initially, this therapy that targets a cancer phenotype $v = 1$has a similar effect as the habitat-dependent therapy. But, in time, the effect is dramatically different. Species 1, whether residing in

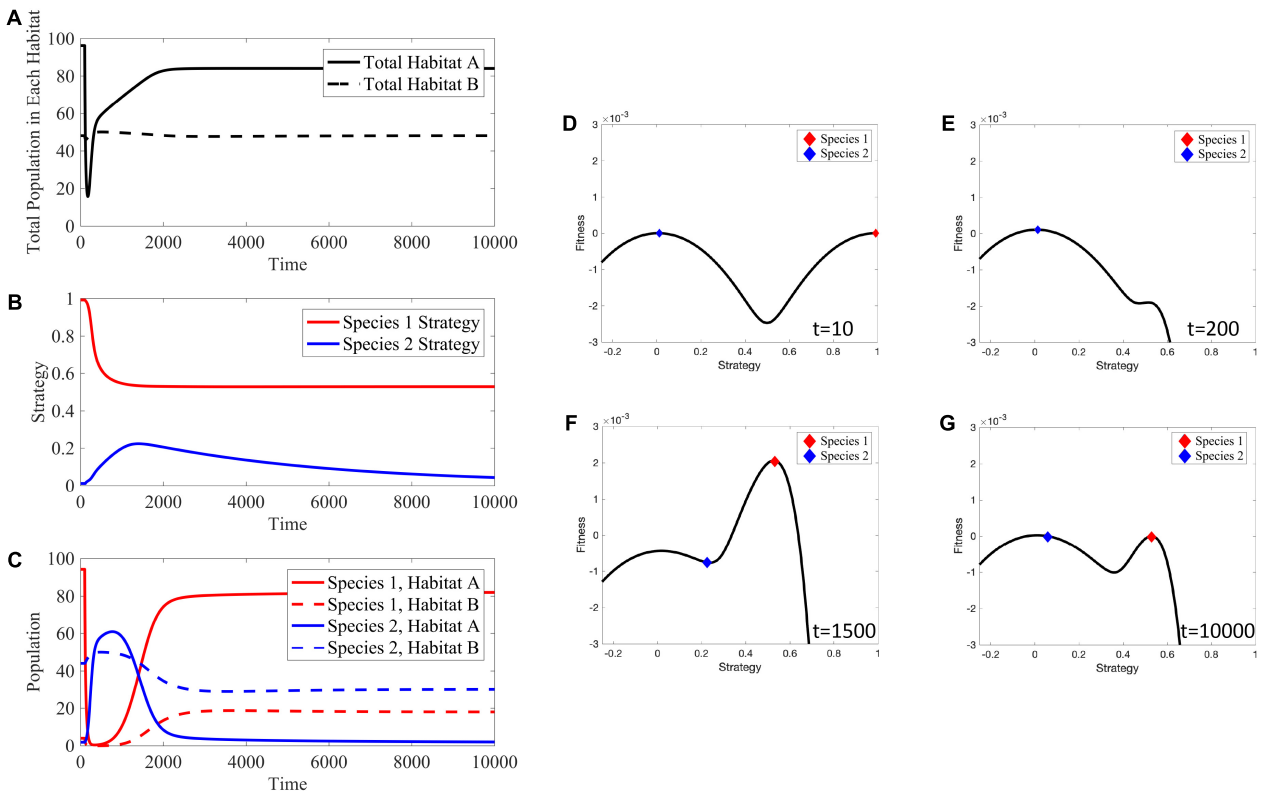


**FIGURE 13 |** Effects of Targeted Therapy. **(A)** The total population in habitat A drastically declines immediately after administration of therapy, but gradually recovers as cells evolve viable trait values. The total population in habitat B stays near its carrying capacity. **(B)** Species 1 evolves a lower strategy to reduce impact of therapy. Species 2 initially evolves a higher strategy in an attempt to occupy the empty niche in habitat A. Once species 1 becomes viable in habitat A, species 2 evolves its strategy back down to near v = 0. **(C)** Transient dynamics: species 1′s population crashes and species 2 attempts to occupy the empty niche in habitat A. Long-term dynamics: species 1 evolves a lower strategy, allowing it to outcompete species 2 in habitat A and allowing species 1 to persist in habitat B at a lower level than species 2. **(D–G)** Depictions of the adaptive landscape over time. Before application of therapy, there are two peaks in the adaptive landscape, corresponding to the two viable habitats. After therapy is administered, the peak corresponding to habitat A shifts toward the one for habitat B; species 1 evolves toward this shifted peak. **Supplementary Movie 4** provides a movie of the panels presented in **(D–G)**.

habitats A or B, can evolve resistance by having a lower strategy value that also has the additional benefit of making species 1 more of a generalist.

Once species 1 evolves a lower strategy, evolutionary rescue is possible and population size recovers. However, note this lower strategy reduces the maximal carrying capacity in habitat A, leading to a lower population equilibrium than prior to treatment. Now, consider species-specific dynamics (**Figures 13B,C**) in which species 1 rapidly declines following therapy. Initially this leaves an open niche in habitat A to which species 2 evolves a higher strategy value. Thus, simultaneously, the strategies of both species 1 and species 2 begin to converge on more generalist phenotypes – species 1 as a form of therapy resistance and species 2 to take advantage of a depopulated habitat A.

Eventually, species 1 evolves into a generalist that allows it to be therapy resistant and to repopulate habitat A though not at the same abundance as pre-treatment. As species 1 recovers, species 2 is again under selection to be a habitat B specialist. Interestingly, at the new post-treatment ESS, species 1′s generalist strategy allows it to have substantial population sizes in both habitats at the expense of species 2. Compared to the pre-treatment ESS, species 2 is still virtually absent from habitat A and resides in habitat B at a reduced population size. While the transient dynamics of the adaptive landscape (**Figures 13D–G**) are dramatic, both the pre- and post-treatment ESSs lead to adaptive landscapes with two peaks. Once therapy is administered, habitat A's peak shifts closer to habitat B's, capturing a trade-off between maximizing carrying capacity in the habitat and avoiding effects of treatment.

The source-sink dynamics led to a counterintuitive result where targeting the phenotype of species 1 actually resulted in an increased number of this cancer cell type as the cancer cells evolved toward a new post-treatment ESS. This result happens because of the strong selection for habitat specialists with or without therapy. If there was only a weak tradeoff between habitat specialists then the pre-treatment system could either have a single generalist species or two specialist species that are not so extreme in their traits values. If the former, then with therapy, the single generalist species would evolve further toward being a habitat B specialist. If the latter, then the habitat A specialist might evolve so far toward the habitat B specialist or vice-versa

that one type would go extinct leaving just a single generalist cancer cell type.

## DISCUSSION

Here, we investigate a relatively unrecognized dynamic in intratumoral evolution – the role of cell migration. Movement of individual cells can have population effects by coupling source-sink habitats, which we show can have profound consequences for tumor biology and treatment. Ongoing intratumoral evolution is frequently described as "branching clonal evolution." That is, cancer cells are subject to genetic mutations and, when a rare mutation results in increased fitness, this new molecular clone expands into an observable population (Greaves and Maley, 2012). However, branching clonal evolution neglects the striking spatial heterogeneity in local environmental conditions, governed primarily by changes in blood perfusion, that are characteristically observed in cancers at macroscopic and microscopic levels. Thus, the selection forces within a tumor will vary considerably. Physically adjacent microscopic regions of a tumor can have dramatically different environmental conditions (Losic et al., 2020).

In contrast to this conventional "mutation-selection" sequence, we propose intratumoral evolution is primarily driven by spatial and temporal variations in environmental conditions. That is, cancer cells in regions of hypoxia and acidosis evolve different phenotypic properties than, for example, those in physiologic environments that may also contain more "predatory" immune cells. This generates frequency- and density-dependent selection within and between tumor microenvironments (Bozic et al., 2012; Soman et al., 2012) produce local cancer cell phenotypes and corresponding genotypes most suited to particular microenvironments – either as generalist or specialist cancer cell types. Thus, mutations that encode phenotypic adaptations suitable for the local environment will become frequent in the extant population. *These genetic changes are consequences of evolution by natural selection, not the cause* (Vincent and Brown, 1988).

Furthermore, a cancer biology paradigm that is difficult to reconcile with evolutionary dynamics is the stem cell hypothesis which posits self-replicating stem cells (Walcher et al., 2020) within a specific niche (Borovski et al., 2011; Oskarsson et al., 2014) give rise to phenotypically variable and non-replicating cells that populate the remainder of the tumor. Evolutionarily, the production of non-replicating daughter cells would be extremely wasteful of scarce resources and likely be subject to negative selection. However, these observed stem cell dynamics could arise from the migration of proliferative and phenotypically distinct cancer cells from source habitats to sink habitats where they adopt a different phenotype and are much less proliferative.

When migration occurs between a source and sink habitat, we demonstrate that, even when the sink habitat cannot maintain a long-term population (e.g., hard sink habitat), it can act as a reservoir of cells that migrate from the source habitat thus maximizing the global population. Furthermore, a harsh sink environment may promote epigenetic modifications (e.g., increased HIF1a expression resulting in upregulation of xenobiotic pathways (Vorrink and Domann, 2014)) that promote resistance to treatment. Thus, the sink habitat may become a source for cells that are also more resistant to subsequent cycles of treatment (Lavi et al., 2013).

In contrast, a soft sink habitat can maintain a small, self-reproducing population. Here, migration from the adjacent source habitat can increase the population of the sink habitat. However, unlike a black hole or hard sink habitat, cells that migrate into a soft sink habitat may proliferate. This is a critical distinction, because proliferation of the migrant cells in the sink habitat is determined by their fitness relative to that of competing native cells. Thus, although the migrant cells are the result of evolutionary selection in the source habitat, their proliferation in the sink habitat is governed by phenotypic adaptation to conditions in the sink habitat. These dynamics can promote "speciation" so that cancer cells even in adjacent tumor regions can have significantly different molecular properties.

Thus, migration of cancer cells between source and sink habitats can produce the clinically observed regional variations in the molecular properties of cancer cells as an alternative to the branching clonal evolution paradigm. Both the spatial variations in the molecular properties of cancer cells in the same tumor at microscopic and macroscopic scales (Gerlinger et al., 2012; Greaves and Maley, 2012; Gerashchenko et al., 2013; Losic et al., 2020) and cancer cell migration (Yamaguchi et al., 2005; Chung et al., 2010; Huang et al., 2011) have been extensively investigated. Yet, to date, no experiments have directly tested the source-sink dynamics described here. Microfluidic co-culture systems may provide an opportunity. There could be adjacent chambers with high (source) and low (soft or hard sink, depending) nutrient availabilities. Hydrogel stiffness could be used to vary migration rates into and out of chambers. Fluorescent labeling could allow for measures of migration, population dynamics, and phenotypic and genotypic changes over time both within and between chambers (Soman et al., 2012; Mi et al., 2016).

We note coupled source-sink habitats may additionally have clinical significance by promoting evolution of resistance following treatment. Thus, while therapy is successful in the source habitat due to increased drug delivery in the case of systemic therapy or improved oxygenation that increases the efficacy of radiation therapy, the source-sink dynamics could reverse after therapy as the surviving cells in the sink habitat become a source, allowing reverse migration and recolonization of the hitherto superior habitat ("rescue effect" in ecology, (Gotelli, 1991)). Adding therapy to the microfluidic co-culture experimental system could address these results.

## CONCLUSION

In conclusion, the ecological and evolutionary dynamics produced by source-sink habitats may provide an underlying explanation to observed spatial variations in genetic and phenotypic properties of cancer cells, an eco-evolutionary foundation for the stem cell paradigm, and suggest critical issues in designing chemotherapeutic and targeted treatment strategies.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

RAG, JSB, and JJC developed the hypothesis. JJC, AB, and JSB developed and analyzed the mathematical models. RAG, RJG, JJC, AB, and JSB wrote and edited the article. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the James S. McDonnell Foundation grant, "Cancer therapy: Perturbing a complex adaptive system," grants from the Jacobson Foundation, NIH/National Cancer Institute (NCI) R01CA170595, Application of Evolutionary Principles to Maintain Cancer Control (PQ21), and NIH/NCI U54CA143970-05 (Physical Science Oncology Network (PSON)) "Cancer as a complex adaptive system". This work has also been supported in part by the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie Grant Agreement No. 690817 and the National Science Foundation Graduate Research Fellowship Program under Grant No. 1746051.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.676071/full#supplementary-material

## REFERENCES

Archetti, M. (2013). Evolutionary game theory of growth factor production: implications for tumour heterogeneity and resistance to therapies. *Br. J. Cancer.* 109, 1056–1062. doi: 10.1038/bjc.2013.336

Borovski, T., De Sousa, E. M. F., Vermeulen, L., and Medema, J. P. (2011). Cancer stem cell niche: the place to be. *Cancer Res.* 71, 634–639.

Boughton, D. A. (1999). Source-sink dynamics in a temporally, heterogeneous environment. *Ecology* 80, 2727–2739.

Bozic, I., Allen, B., and Nowak, M. A. (2012). Dynamics of targeted cancer therapy. *Trends Mol. Med.* 18, 311–316.

Brown, J. S., and Pavlovic, N. (1992). Evolution in heterogeneous environments: effects of migration on habitat specialization. *Evol. Ecol.* 6, 360–382. doi: 10.1007/bf02270698

Chung, S., Sudo, R., Vickerman, V., Zervantonakis, I. K., and Kamm, R. D. (2010). Microfluidic platforms for studies of angiogenesis, cell migration, and cell–cell interactions. *Ann. Biomed. Eng.* 38, 1164–1177. doi: 10.1007/s10439-010-9899-3

Cohen, Y. V. T., and Brown, J. S. (1999). A G-function approach to fitness minima, fitness maxima, evolutionarily stable strategies and adaptive landscapes. *Evol. Ecol. Res.* 1, 923–942.

Cure, K., Thomas, L., Hobbs, J. A., Fairclough, D. V., and Kennington, W. J. (2017). Genomic signatures of local adaptation reveal source-sink dynamics in a high gene flow fish species. *Sci. Rep.* 7:8618.

Diffendorfer, J. E. (1998). Testing models of source-sink dynamics and balanced dispersal. *Oikos* 81, 417–433. doi: 10.2307/3546763

Dongre, A., and Weinberg, R. A. (2019). New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat. Rev. Mol. Cell Biol.* 20, 69–84. doi: 10.1038/s41580-018-0080-4

Fisher, R., Larkin, J., and Swanton, C. (2012). Inter and intratumour heterogeneity: a barrier to individualized medical therapy in renal cell carcinoma? *Front. Oncol.* 2:49. doi: 10.3389/fonc.2012.00049

Fu, F., Nowak, M. A., and Bonhoeffer, S. (2015). Spatial heterogeneity in drug concentrations can facilitate the emergence of resistance to cancer therapy. *PLoS Comput. Biol.* 11:e1004142. doi: 10.1371/journal.pcbi.1004142

Gerashchenko, T. S., Denisov, E. V., Litviakov, N. V., Zavyalova, M. V., Vtorushin, S. V., Tsyganov, M. M., et al. (2013). Intratumor heterogeneity: nature and biological significance. *Biochemistry (Mosc)* 78, 1201–1215. doi: 10.1134/s0006297913110011

Geritz, S. A. H., Kisdi, E'., Mesze'NA, G., and Metz, J. A. J. (1998). Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. *Evol. Ecol.* 12, 35–57. doi: 10.1023/A:1006554906681

Gerlinger, M., Rowan, A. J., Horswell, S., Math, M., Larkin, J., Endesfelder, D., et al. (2012). Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* 366, 883–892. doi: 10.1056/NEJMoa1113205

Gotelli, N. J. (1991). Metapopulation models: the rescue effect, the propagule rain, and the core-satellite hypothesis. *Am. Nat.* 138, 768–776. doi: 10.1086/285249

Gravel, D., Guichard, F., Loreau, M., and Mouquet, N. (2010). Source and sink dynamics in meta-ecosystems. *Ecology.* 91, 2172–2184. doi: 10.1890/09-0843.1

Greaves, M., and Maley, C. C. (2012). Clonal evolution in cancer. *Nature.* 481, 306–313.

Hinohara, K., and Polyak, K. (2019). Intratumoral heterogeneity: more than just mutations. *Trends Cell Biol.* 29, 569–579. doi: 10.1016/j.tcb.2019.03.003

Holt, R. (1985). Population-dynamics in 2-patch environments - some anomalous consequences of an optimal habitat distribution. *Theor. Popul. Biol.* 28, 181–208. doi: 10.1016/0040-5809(85)90027-9

Holt, R. D., and Gomulkiewicz, R. (1997). How does immigration influence local adaptation? A reexamination of a familiar paradigm. *Am. Nat.* 149, 563–572. doi: 10.1086/286005

Holt, R. D., Barfield, M., and Gomulkiewicz, R. (2004). Temporal variation can facilitate niche evolution in harsh sink environments. *Am. Nat.* 164, 187–200. doi: 10.2307/3473438

Huang, Y., Agrawal, B., Sun, D., Kuo, J. S., and Williams, J. C. (2011). Microfluidics-based devices: new tools for studying cancer and cancer stem cell migration. *Biomicrofluidics* 5:013412. doi: 10.1063/1.3555195

Jardim-Perassi, B. V., Huang, S., Dominguez-Viqueira, W., Poleszczuk, J., Budzevich, M. M., Abdalah, M. A., et al. (2019). Multiparametric MRI and coregistered histology identify tumor habitats in breast cancer mouse models. *Cancer Res.* 79, 3952–3964. doi: 10.1158/0008-5472.can-19-0213

Johnson, D. M. (2004). Source-sink dynamics in a temporally, heterogeneous environment. *Ecology* 85, 2037–2045. doi: 10.1890/03-0508

Johnson, K. E., Howard, G., Mo, W., Strasser, M. K., Lima, E., Huang, S., et al. (2019). Cancer cell population growth kinetics at low densities deviate from the exponential growth model and suggest an Allee effect. *PLoS Biol.* 17:e3000399. doi: 10.1371/journal.pbio.3000399

Klein, C. A., Blankenstein, T. J., Schmidt-Kittler, O., Petronio, M., Polzer, B., Stoecklein, N. H., et al. (2002). Genetic heterogeneity of single disseminated tumour cells in minimal residual cancer. *Lancet.* 360, 683–689. doi: 10.1016/s0140-6736(02)09838-0

Lavi, O., Greene, J. M., Levy, D., and Gottesman, M. M. (2013). The role of cell density and intratumoral heterogeneity in multidrug resistance. *Cancer Res.* 73, 7168–7175. doi: 10.1158/0008-5472.can-13-1768

Li, F., Tiede, B., Massague, J., and Kang, Y. (2007). Beyond tumorigenesis: cancer stem cells in metastasis. *Cell Res.* 17, 3–14. doi: 10.1038/sj.cr.7310118

Lloyd, M. C., Cunningham, J. J., Bui, M. M., Gillies, R. J., Brown, J. S., and Gatenby, R. A. (2016). Darwinian dynamics of intratumoral heterogeneity: not solely random mutations but also variable environmental selection forces. *Cancer Res.* 76, 3136–3144. doi: 10.1158/0008-5472.can-15-2962

Losic, B., Craig, A. J., Villacorta-Martin, C., Martins-Filho, S. N., Akers, N., Chen, X., et al. (2020). Intratumoral heterogeneity and clonal evolution in liver cancer. *Nat Commun.* 11:291.

Mi, S., Du, Z., Xu, Y., Wu, Z., Qian, X., Zhang, M., et al. (2016). Microfluidic co-culture system for cancer migratory analysis and anti-metastatic drugs screening. *Sci. Rep.* 6, 1–11.

Moreno-Gamez, S., Hill, A. L., Rosenbloom, D. I., Petrov, D. A., Nowak, M. A., and Pennings, P. S. (2015). Imperfect drug penetration leads to spatial monotherapy and rapid evolution of multidrug resistance. *Proc. Natl. Acad. Sci.U.S.A.* 112, E2874–E2883.

Morris, D. W. (1991). On the evolutionary stability of dispersal to sink habitats. *Am. Nat.* 137, 907–911. doi: 10.1086/285200

Oskarsson, T., Batlle, E., and Massague, J. (2014). Metastatic stem cells: sources, niches, and vital pathways. *Cell Stem Cell.* 14, 306–321. doi: 10.1016/j.stem.2014.02.002

Paul, C. D., Mistriotis, P., and Konstantopoulos, K. (2017). Cancer cell motility: lessons from migration in confined spaces. *Nat. Rev. Cancer* 17, 131–140. doi: 10.1038/nrc.2016.123

Perez-Velazquez, J., and Rejniak, K. A. (2020). Drug-induced resistance in micrometastases: analysis of spatio-temporal cell lineages. *Front. Physiol.* 11:319. doi: 10.3389/fphys.2020.00319

Polacheck, W. J., Zervantonakis, I. K., and Kamm, R. D. (2013). Tumor cell migration in complex microenvironments. *Cell Mol. Life Sci.* 70, 1335–1356. doi: 10.1007/s00018-012-1115-1

Pulliam, H. R. (1988). Sources, sinks, and population regulation. *Am. Nat.* 132, 652–661. doi: 10.1086/284880

Quinn, J. J., Jones, M. G., Okimoto, R. A., Nanjo, S., Chan, M. M., Yosef, N., et al. (2021). Single-cell lineages reveal the rates, routes, and drivers of metastasis in cancer xenografts. *Science* 371, eabc1944. doi: 10.1126/science.abc1944

Rosenzweig, M. L. (1981). A theory of habitat selection. *Ecology* 62, 327–335.

Ruud, K. F., Hiscox, W. C., Yu, I., Chen, R. K., and Li, W. (2020). Distinct phenotypes of cancer cells on tissue matrix gel. *Breast Cancer Res.* 22:82.

Schmidt, K. A., Earnhardt, J. M., Brown, J. S., and Holt, R. D. (2000). Habitat selection under temporal heterogeneity: exorcizing the ghost of competition past. *Ecology.* 81, 2622–2630. doi: 10.1890/0012-9658(2000)081[2622:hsuthe]2.0.co;2

Scott, J., and Marusyk, A. (2017). Somatic clonal evolution: a selection-centric perspective. *Biochim. Biophys. Acta* 1867, 139–150. doi: 10.1016/j.bbcan.2017.01.006

Soman, P., Kelber, J. A., Lee, J. W., Wright, T. N., Vecchio, K. S., Klemke, R. L., et al. (2012). Cancer cell migration within 3D layer-by-layer microfabricated photocrosslinked PEG scaffolds with tunable stiffness. *Biomaterials* 33, 7064–7070. doi: 10.1016/j.biomaterials.2012.06.012

Staneva, R., El Marjou, F., Barbazan, J., Krndija, D., Richon, S., Clark, A. G., et al. (2019). Cancer cells in the tumor core exhibit spatially coordinated migration patterns. *J. Cell Sci.* 132: jcs220277.

Tarjuelo, R., Traba, J., Morales, M. B., and Morris, D. W. (2017). Isodars unveil asymmetric effects on habitat use caused by competition between two endangered species. *Oikos* 126, 73–81. doi: 10.1111/oik.03366

Te Boekhorst, V., Preziosi, L., and Friedl, P. (2016). Plasticity of cell migration in vivo and in silico. *Annu. Rev. Cell Dev. Biol.* 32, 491–526. doi: 10.1146/annurev-cellbio-111315-125201

Vincent, T. L., and Brown, J. (1988). The evolution of ESS theory. *Ann. Rev. Ecol. Syst.* 19, 423–443.

Vincent, T. L., and Brown, J. (2002). Evolutionarily stable strategies in multistage biological systems. *Selection* 2, 85–102. doi: 10.1556/select.2.2001.1-2.7

Vincent, T. L., and Brown, J. S. (2005). *Evolutionary Game Theory, Natural Selection, and Darwinian Dynamics*. Cambridge, NY: Cambridge University Press.

Vincent, T. L., Cohen, Y., and Brown, J. S. (1993). Evolution via strategy dynamics. *Theor. Popul. Biol.* 44, 149–176. doi: 10.1006/tpbi.1993.1023

Vorrink, S. U., and Domann, F. E. (2014). Regulatory crosstalk and interference between the xenobiotic and hypoxia sensing pathways at the AhR-ARNT-HIF1alpha signaling node. *Chem. Biol. Interact.* 218, 82–88. doi: 10.1016/j.cbi.2014.05.001

Walcher, L., Kistenmacher, A. K., Suo, H., Kitte, R., Dluczek, S., Strauss, A., et al. (2020). Cancer stem cells-origins and biomarkers: perspectives for targeted personalized therapies. *Front. Immunol.* 11:1280. doi: 10.3389/fimmu.2020.01280

Wang, H. F., Warrier, T., Farran, C. A., Zheng, Z. H., Xing, Q. R., Fullwood, M. J., et al. (2020). Defining essential enhancers for pluripotent stem cells using a features-oriented crispr-cas9 screen. *Cell Rep.* 33:108309. doi: 10.1016/j.celrep.2020.108309

Winker, K. (2000). Migration and speciation. *Nature* 404:36.

Yamaguchi, H., Wyckoff, J., and Condeelis, J. (2005). Cell migration in tumors. *Curr. Opin. Cell Biol.* 17, 559–564. doi: 10.1016/j.ceb.2005.08.002

Zhang, X., Peng, Y., Li, C., Li, Q., Yu, Z., Pang, Y., et al. (2019). Genomic heterogeneity and branched evolution of early stage primary acral melanoma shown by multiregional microdissection sequencing. *J. Invest. Dermatol.* 139, 1526–1534. doi: 10.1016/j.jid.2019.01.019

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past collaboration with one of the authors, JB.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 23 July 2021
doi: 10.3389/fevo.2021.675638

# Paracrine Behaviors Arbitrate Parasite-Like Interactions Between Tumor Subclones

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Explaining the emergence and maintenance of intratumor heterogeneity is an important question in cancer biology. Tumor cells can generate considerable subclonal diversity, which influences tumor growth rate, treatment resistance, and metastasis, yet we know remarkably little about how cells from different subclones interact. Here, we confronted two murine mammary cancer cell lines to determine both the nature and mechanisms of subclonal cellular interactions *in vitro*. Surprisingly, we found that, compared to monoculture, growth of the "winner" was enhanced by the presence of the "loser" cell line, whereas growth of the latter was reduced. Mathematical modeling and laboratory assays indicated that these interactions are mediated by the production of paracrine metabolites resulting in the winner subclone effectively "farming" the loser. Our findings add a new level of complexity to the mechanisms underlying subclonal growth dynamics.

**Keywords: cancer evolution, intratumor clonal heterogeneity, evolutionary game theory, parasitism, paracrine signaling, beta-hydroxybutirate, lactate, Lotka–Volterra model**

OPEN ACCESS

***Edited by:***
*Sarah R. Amend, Johns Hopkins Medicine, United States*

***Reviewed by:***
*Robert Beckman, Georgetown University, United States*
*David Basanta, Moffitt Cancer Center, United States*

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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *05 March 2021*
***Accepted:*** *28 June 2021*
***Published:*** *23 July 2021*

***Citation:***
*Noble RJ, Walther V, Roumestand C, Hochberg ME, Hibner U and Lassus P (2021) Paracrine Behaviors Arbitrate Parasite-Like Interactions Between Tumor Subclones. Front. Ecol. Evol. 9:675638. doi: 10.3389/fevo.2021.675638*

## INTRODUCTION

Considering tumors as complex ecosystems has led to the notion that diverse cancer cell subclones engage in heterotypic interactions reminiscent of those that operate in organismal communities (Heppner, 1984; Axelrod et al., 2006; Merlo et al., 2006; Tabassum and Polyak, 2015). Mutually negative interactions are thought to be ubiquitous in cancer (Nowell, 1976; Greaves and Maley, 2012). As in classic ecosystems, cancer cells compete for nutrients and space, and competition between emergent subclones gives rise to complex temporal and spatial dynamics of tumor composition and growth (Tabassum and Polyak, 2015). Positive ecological interactions (mutualism and commensalism) have been observed in cancer models in mice (Calbo et al., 2011; Cleary et al., 2014) and in drosophila (Ohsawa et al., 2012). In these cases, one subclone acquires new abilities, such as the capacity to grow or metastasize, only in the presence of another subclone, resulting in the tumor as a whole progressing toward a more aggressive phenotype. In contrast, the prevalence within tumors of asymmetric interactions such as amensalism, parasitism and facilitation remains an open question. Defining the mechanisms of tumor ecology is essential for a better understanding

of cancer progression and may lead to novel therapeutic strategies (Gatenby and Brown, 2017; Maley et al., 2017).

To gain insight into molecular and cellular events related to ecological interactions between cancer subclones, we took advantage of a model described over three decades ago, based on two closely related murine cancer cell lines derived from a single spontaneous mouse mammary tumor (Dexter et al., 1978; Miller et al., 1988). When cultured separately, the two cell lines have similar growth rates, yet in co-culture one cell line (the "winner") expands at the expense of the other (the "loser"). Our careful re-examination of this model, combining experiments with mathematical modeling and parameter inference, indicated that the cellular behaviors of the two subclones are surprisingly sophisticated. Both cell lines produce paracrine metabolites that boost proliferation of the winner and also decrease the growth rate of the loser. Our results thus unveil a type of facultative parasitic behavior of the winner subclone. We further identified beta-hydroxybutyrate and lactate as metabolites that contribute to these phenotypes and characterized their modes of action. We discuss our results in the context of how previously underappreciated ecological interactions may contribute to the complexity of tumor growth dynamics.

# RESULTS

## 4T07 Cells Have a "Winner" Phenotype

Two cell lines derived from a single mouse mammary carcinoma – 168 and 4T07 cells – have similar growth rates when cultured individually, yet the 4T07 clone displays a dominant phenotype when grown together, either in cell culture or in orthotopic allografts *in vivo* (Miller et al., 1988). Several hypotheses to account for this interesting behavior had been tested in the original work, but the precise mechanism behind these competitive interactions has so far not been identified.

We began by verifying that in our hands the lines maintain their competitive characteristics. To facilitate lineage tracing we first generated lines stably expressing GFP, the expression of which did not alter cell growth (**Figure 1A**). Next, we followed growth characteristics of 4T07 and 168FARN cells, the latter being a drug-resistant derivative of the original 168 clone (Aslakson et al., 1991), in a continuous culture for 3 weeks. The cells were seeded as 1:1 mix at a density that allowed them to reach confluence within 3–4 days, at which point they were harvested and re-seeded in a new well at the original density. Remaining cells were analyzed by flow cytometry to determine the proportion of GFP expressing clones in the expanding population.

The homotypic co-culture (same line with and without GFP) confirmed that GFP has no impact on cellular proliferation (**Figures 1B**, **2B**). In contrast, heterotypic co-culture conditions (two different lines, one expressing GFP) revealed the dominance of the 4T07 clone (**Figures 1B**, **2B**).

These results confirm the originally described ecological interaction between the clones: 4T07 gradually dominates the culture while the 168FARN cells become scarce within 15–17 days. Importantly, the dominant phenotype is independent of the starting ratio between the two cell lines (**Supplementary Figures 1A,B**).

## Co-culture Alters the Proliferation Rates of Both "Winner" and "Loser" Cells

As originally discussed for the two clones under study (Miller et al., 1988), the expansion of a single clone in co-culture could be due to alterations in cell death or changes in the proliferation rates of either or both clones. We measured apoptosis in the loser 168FARN clone and found identical, very low levels of cell death under homotypic and heterotypic conditions (**Supplementary Figure 2A**). Next, we used time-lapse microscopy to assess the growth dynamics of both clones in continuous culture. The cells were seeded at a density that allowed reaching confluence in 4 days and were photographed every 45 min for the last 3 days. We measured the overall pixel intensity for each frame (**Figure 3A**) as a proxy for the growth rate of the fluorescently tagged cell line. This analysis revealed that under co-culture conditions, the growth rate of 168FARN decreased, whereas that of 4T07 increased relative to mono-cultures. To test whether increased net growth of the winner population is due to the alteration of proliferation, we estimated the proportion of cells in the S phase of the cell cycle by performing pulse-chase EdU staining. The results presented in **Supplementary Figure 2B** confirmed that heterotypic co-culture gave rise to significant decrease in cells actively replicating DNA for the loser clone and a significant increase in the winner clone. Overall, these results suggest that the dominant phenotype displayed by the winner cells in co-culture can be explained by changes in proliferation that operate in opposing directions on the winner and the loser cells.

## Mathematical Modeling and Inference of Evolutionary Parameter Values

To gain further insight into the ecological interactions between the winner and loser cell types we turned to mathematical modeling. Examination of the growth curves revealed two distinct phases of evolutionary dynamics (**Figures 3A,B**). In phase 1, from 0 to 45 h, the two cell types grew exponentially in both homotypic and heterotypic cultures, and the growth rate of 168 was higher than that of 4T07. This first phase can be regarded as a transition period before the cells start altering and responding to their new environment. By contrast in phase 2, from 45 to 72 h, the growth curves were strongly affected by interactions within and between the two cell types, and 4T07 grew faster than 168. To enable us to determine the mode of the ecological dynamics in each phase, we opted for a parsimonious, piecewise mathematical model. Specifically, we assumed a model with exponential growth in phase 1 and a transition to density-dependent competitive Lotka–Volterra-type dynamics in phase 2.

By fitting our model to the homotypic growth curves, we inferred the values of the phase 1 and phase 2 growth rates and the within-type interaction parameters (see section "Materials and Methods"). To infer the between-type interactions, we used additional data from 72-h competition assays, covering a wide range of initial ratios of the two cell types. Although this latter

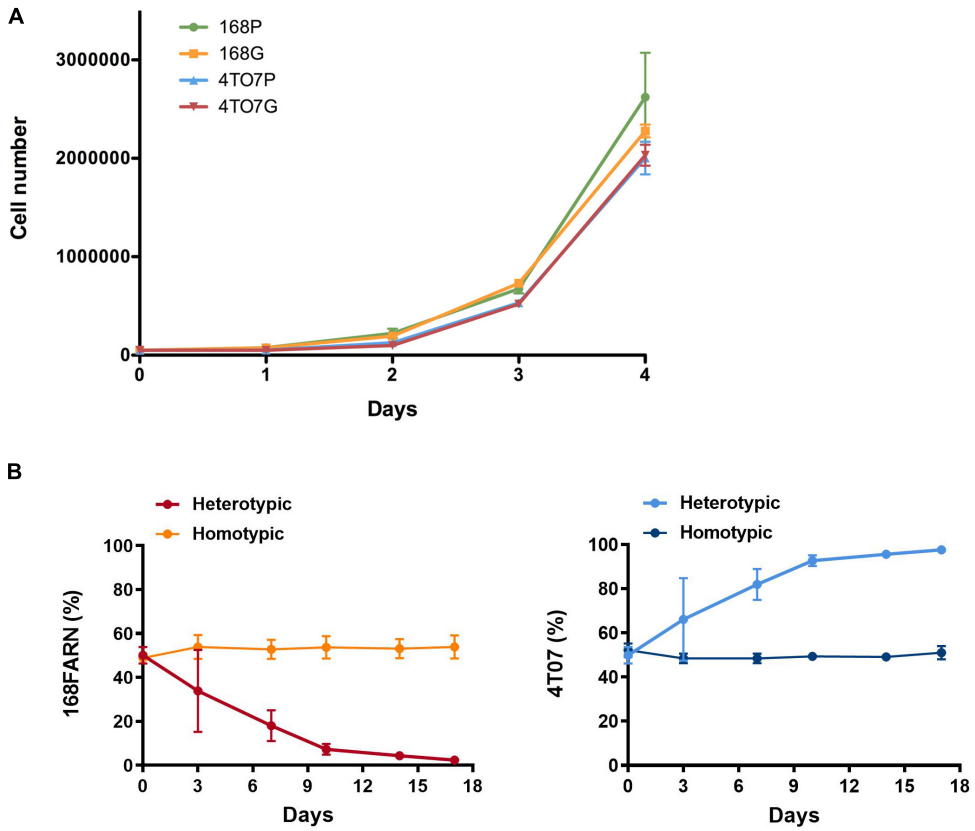


**FIGURE 1 |** Mutual impacts on subclonal growth. **(A)** 168FARN and 4T07 parental cells were transduced either with an empty retroviral vector (168P and 4T07P) or with labeled with a GFP-encoding retrovirus (168G and 4T07G). Cells were seeded in triplicate in six-well plates at a density of 50,000 cells/well and cultured for the indicated times before harvesting and counting. **(B)** $10^5$ Cells were seeded at a 1:1 ratio in homotypic (parental and GFP expressing derivative of the same cell line) or heterotypic (different cell lines, one expressing GFP) co-cultures and harvested and replated at the initial densities ($10^5$ cells/plate) at indicated times. The ratios of GFP-labeled to unlabeled cells were estimated by flow cytometry. The results represent data from three independent experiments and are shown as mean ± SEM.

data set comprises only the initial and final proportions (at the beginning of phase 1 and the end of phase 2), we were able to infer the proportions at the beginning of phase 2 by adjusting for the exponential growth of both types during phase 1. We then used these inferred proportions and our previously inferred parameter values to estimate the remaining interaction parameters (see section "Materials and Methods"). The resulting model gives a good fit to the competition assay data (**Figure 2A**, first column) and is consistent with heterotypic time-lapse data not used for parameter inference (**Figure 3** and **Supplementary Figure 6**).

The inferred parameter values (**Table 1**) imply that during phase 2, 4T07 has a large negative effect on both itself and on 168, consistent with 4T07 producing a harmful diffusible factor. The negative effect of 168 on itself is only about half as large, and 168 has approximately zero net effect on the growth of 4T07. This suggests that ubiquitous negative effects of 168 on 4T07 (e.g., likely due to waste products and competition for resources) are offset by positive effects, such as due to a beneficial diffusible factor. Also, during phase 2, the intrinsic growth rate of 168 (that is, the inferred growth rate before accounting for cell–cell interactions) is approximately 30% lower than that of 4T07, consistent with the conventional hypothesis that producing beneficial factors is costly. This disadvantage is offset by 168 having an approximately 30% higher carrying capacity (defined as the upper limit of the homotypic population size). Over phase 2, or any longer period that includes phase 2, the inferred net growth rate of 4T07 (that is, the growth rate after accounting for cell–cell interactions) is invariably higher than that of 168, which means 4T07 will come to dominate numerically, no matter their initial frequency.

Since we also conducted 96-h competition assays, we were able to infer the population dynamics during a third phase (72–96 h). For every initial ratio of the two cell types, the growth rate difference (also known as the gain function) was on average lower in the 96-h than in 72-h competition assays (**Supplementary Figure 5**). Moreover, this difference did not depend on the initial ratio, which implies it was not caused by a change in interaction parameters. A parsimonious way to account for this effect is to assume a reduction in 4T07's intrinsic growth rate during phase 3, as would be expected to result from starvation and/or the build-up of toxic waste products. Making this adjustment to our model indeed produces a better fit to the competition assay data (**Figure 2A**, middle column, **Figures 2B,C**). The predicted dynamics are shown in **Figures 3C,D**.

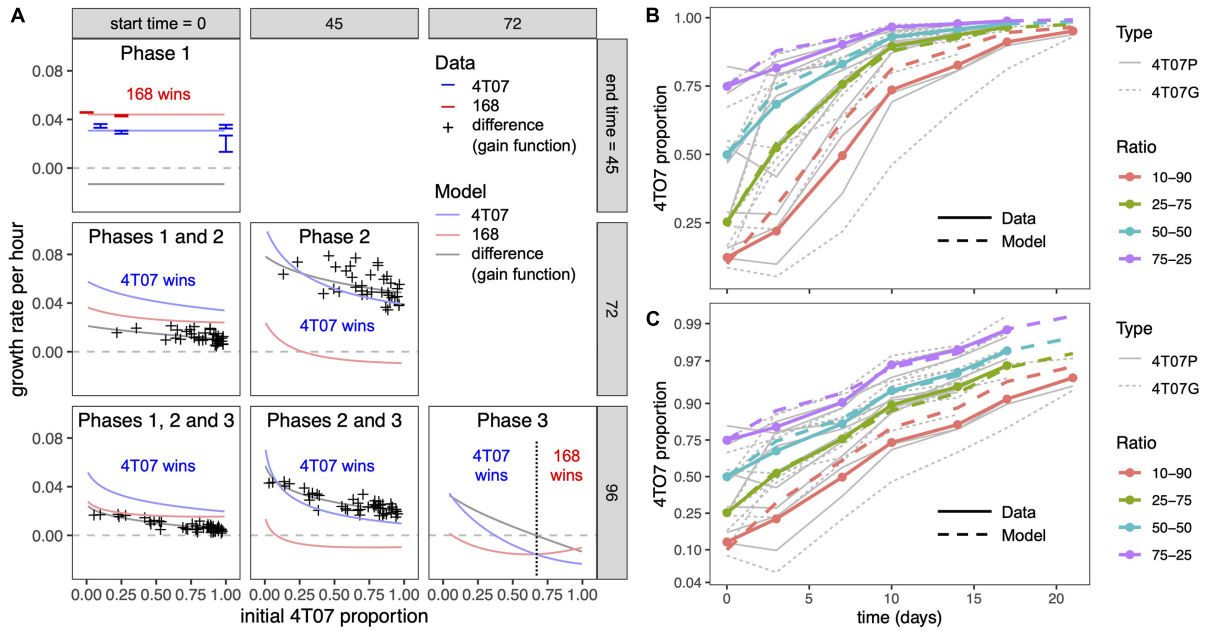


**FIGURE 2 |** Mean net growth rate differences according to mathematical model and experimental data. **(A)** Inferred mean net growth rates and mean net growth rate differences (gain functions) over different time periods, corresponding to different phases within competition assays. Columns correspond to different start times and rows to different end times of the phase(s) under consideration. For example, the center panel labeled "Phase 2" corresponds to the period between 45 and 72 h. The initial 4T07 proportion (horizontal axis) is measured at the start of the respective period and the growth rate (vertical axis) is averaged over the period. Phase 1 data are from time-lapse microscopy. Other data points in the first column are from serial competition assays, such that each point corresponds to the slope of a thin gray line in panel **(B)**. Data points in the middle column are obtained from the competition assay data by adjusting for exponential growth during phase 1 (see section "Materials and Methods"). Curves are the results of our mathematical model (see section "Materials and Methods") with parameter values inferred from data (**Table 1**). **(B)** 4T07 frequency dynamics across serial competition assays. Thick solid lines are averaged data (means of replicates with similar initial 4T07 proportions) and thick dashed lines are results of our mathematical model with parameter values inferred from data. Thin gray lines are data for individual experiments. A total of $10^5$ cells were seeded in co-cultures and harvested and replated as indicated. 4T07 parental cells were transduced either with an empty retroviral vector (4T07P) or labeled with a GFP-encoding retrovirus (4T07G). The ratios of GFP to unlabeled cells were estimated by flow cytometry. **(C)** Logit-transformed 4T07 frequency dynamics. This panel shows the same data as panel **(B)** but with a logit-transformed vertical axis so that the slope of each curve is equal to the mean net growth rate difference (the gain function, as described in section "Materials and Methods" and **Supplementary Figure 7**).

Finally, having inferred all the evolutionary parameter values, we calculated net growth rates of the two cell types, averaged over different time periods. Over any period that includes phase 2, our model predicts that the net growth rate of both cell types will decrease non-linearly with increasing initial 4T07 frequency (pink and blue curves in **Figure 2A**). However, the net growth rate of 4T07 decreases faster than that of 168, which is why the gain function (gray curve in **Figure 2A**) also decreases. In phase 3, if the initial proportion of 4T07 is high (above 70%), then 168 has a higher net growth rate than 4T07, but in this case both of the inferred net growth rates are negative. Overall, the interactions are effectively equivalent to those of a parasite and its host, such that the "loser" 168 suffers from the presence of the "winner" 4T07, while also enhancing the winner's fitness.

## β-Hydroxybutyrate Secreted by the Loser Clone Stimulates Winner Clone Proliferation

To identify the molecular mechanisms at the basis of the altered growth of winners and losers when in co-culture, we first focused on the increase in proliferation rate of 4T07 cells. Heterotypic culture experiments performed at low cell density suggested that the dominant effect did not require extensive cell–cell contacts (**Supplementary Figure 3**). We reasoned that a soluble factor secreted by 168FARN could induce a proliferation boost in 4T07. To test this hypothesis, we collected conditioned media from each line cultured for 3 days and used each medium separately to grow 4T07 for an additional 24 h. As controls, we either left the 4T07 medium after the 3 days of conditioning or replaced it with fresh medium. The results shown in **Figure 2A** confirm our hypothesis: the medium conditioned by 168FARN induced a significant increase in 4T07 proliferation. Importantly, this effect was not due to differences of medium exhaustion by the two cell lines, since the addition of fresh medium did not boost 4T07 proliferation.

Since our data strongly suggested that a soluble factor originating from 168FARN accounted for the increase in 4T07 proliferation, we next sought to define its molecular nature. First, we separated the 168FARN-conditioned medium into high and low MW fractions with a 3 KDa molecular cutoff column. The low MW fraction contains mainly metabolites while the high one is enriched in proteins. After complementing each fraction, respectively, with 10% serum or with DMEM to obtain full

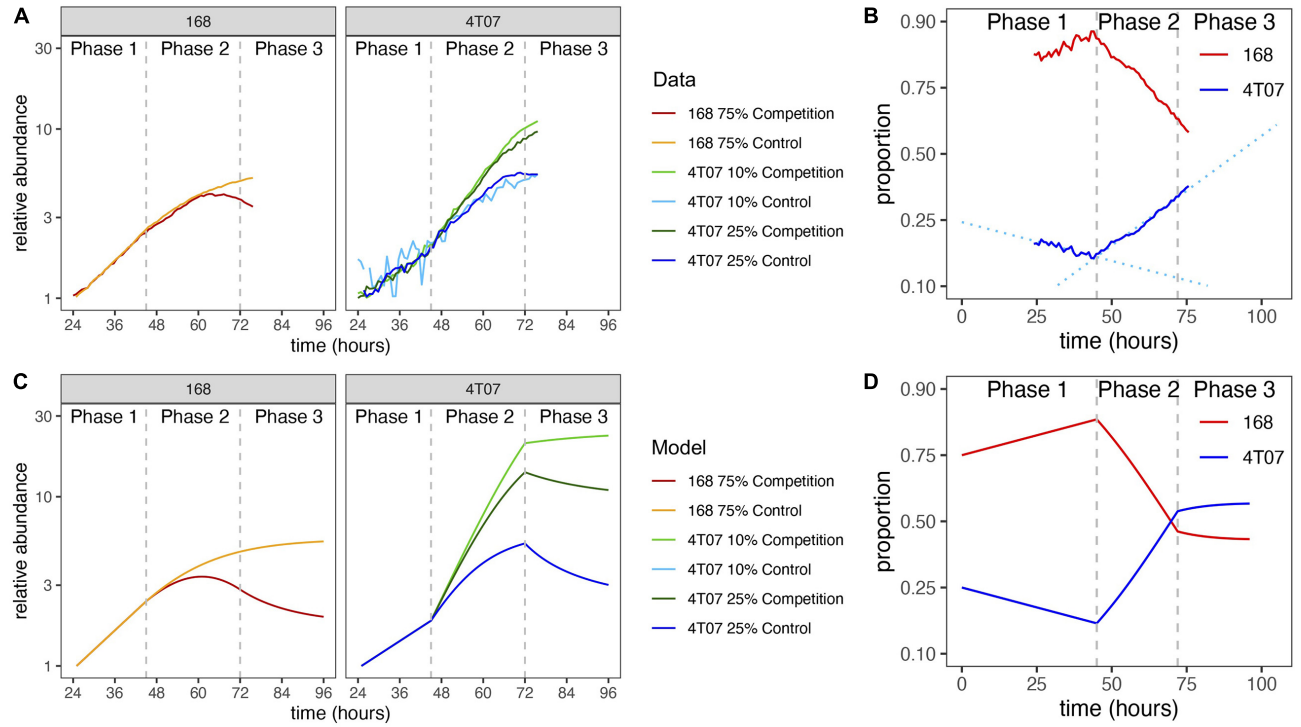


**FIGURE 3 |** Normalized growth curves of homotypic and heterotypic mixes of subclones. **(A)** The GFP fluorescence of the labeled subclone was measured by time-lapse microscopy. Cultures were seeded with $10^5$ cells per well. Log-transformed data were normalized by fitting regression lines and dividing by the inferred value at 24 h. Vertical dashed lines mark the start of phase 2 (45 h) and phase 3 (72 h). **(B)** Frequency dynamics. Curves obtained by combining the results of two competition experiments: one with labeled 4T07 and the other with labeled 168. The initial 4T07 proportion was 25% in both cases. The vertical axis is logit-transformed so that the slope of each curve is equal to the difference in net growth rates at the corresponding time (see section "Materials and Methods"). Dotted regression lines are shown to draw attention to the change of slope. **(C)** Normalized growth curves according to mathematical model with parameter values inferred from data. The model is described in section "Materials and Methods" and parameter values are given in **Table 1**. **(D)** Frequency dynamics according to mathematical model with parameter values inferred from data.

media conditioned with either low or high MW secretomes, we used them in a proliferation assay as in **Figure 4A**. The results (**Figure 4B**) of this series of experiments unambiguously identified the low MW fraction of the 168FARN-conditioned medium as the source of the pro-proliferative factor. To further explore its identity, we employed nuclear magnetic resonance spectroscopy to compare the composition of low MW fractions prepared from fresh medium and from the 168FARN- and 4T07-conditioned ones (Henke et al., 1996). Two major peaks specific for the conditioned media corresponded to a very strong signal for lactate secreted by 4T07 cells, and a significant increase in a peak identified as β-hydroxybutyrate (BHB) in the 168FARN-conditioned medium (**Figure 5A**). BHB is a ketone body mainly produced by the liver after long fasting periods and which is used by different tissues as a source of carbon to supplement the lack of glucose (Newman and Verdin, 2017). In addition, BHB is also produced by other cell types, such as adipocytes or cancer cells (Grabacka et al., 2016; Huang et al., 2017; Wang et al., 2017). To confirm the NMR-based identification of the BHB peak, we employed an enzymatic assay to measure BHB concentration in conditioned media from 4T07 and 168FARN (**Figure 5B**). The results were in perfect agreement with the NMR analysis: BHB production is significantly higher in the loser than in the winner cell clone. To test whether this metabolite was indeed responsible for the increased proliferation of 4T07, we next complemented the medium of exponentially growing 4T07 cells with purified BHB. As shown in **Figure 5C**, BHB increased the 4T07 proliferation rate to a level comparable to that obtained with the 168-conditioned medium. We thus conclude that loser cells increase the winner's growth rate through the secretion of BHB.

## Presence of the Winner Clone Stimulates β-Hydroxybutyrate Production by Loser Cells

After assessing BHB production in homotypic cell culture, we evaluated its secretion under heterotypic conditions. We grew 168FARN alone or together with 4T07 at a 1:1 ratio, maintaining the overall cell density constant. Surprisingly, despite the fact that under heterotypic conditions there are at least 50% fewer loser cells (which are the main producers of BHB, cf. **Figure 5B**), the overall level of secreted BHB was higher than in the homotypic culture (**Figure 5D**). This suggests that either the presence of 4T07 increased the production of the metabolite by 168FARN or, alternatively, that it was 4T07 that produced more metabolite

**TABLE 1 |** Mathematical model parameter values inferred from data.

| Parameter | Phase(s) | Inferred value | Interpretation |
|---|---|---|---|
| $r_{L,1}$ | 1 | 0.044 | 168 growth rate in phase 1 (per hour) |
| $r_{W,1}$ | 1 | 0.031 | 4T07 growth rate in phase 1 (per hour) |
| $r_{L,2}$ | 2 and 3 | 0.073 | 168 intrinsic growth rate in phase 2 (per hour) |
| $r_{W,2}$ | 2 | 0.102 | 4T07 intrinsic growth rate in phase 2 (per hour) |
| $r_{W,3}$ | 3 | 0.04 | 4T07 intrinsic growth rate in phase 3 (per hour) |
| $a$ | 2 and 3 | −0.004 | Density-dependent effect of 168 on 168 |
| $b$ | 2 and 3 | −0.010 | Density-dependent effect of 4T07 on 168 |
| $c$ | 2 and 3 | 0.000 | Density-dependent effect of 168 on 4T07 |
| $d$ | 2 and 3 | −0.008 | Density-dependent effect of 4T07 on 4T07 |
| $K_L = -r_{L,2}/a$ | 2 and 3 | 17 | 168 carrying capacity, relative to initial population size |
| $K_W = -r_{W,2}/d$ | 2 | 13 | 4T07 carrying capacity, relative to initial population size |
| $\beta = b/a$ | 2 and 3 | 2.4 | Effect of 4T07 on 168, relative to effect of 168 on 168 |
| $\gamma = c/d$ | 2 and 3 | 0.0 | Effect of 168 on 4T07, relative to effect of 4T07 on 4T07 |

*The interaction terms a, b, c, and d are relative to population size, which is, in turn, relative to initial population size.*

when grown in the presence of 168FARN. To distinguish between these hypotheses, we cultured both lines individually for 3 days, measured BHB concentration, and then exchanged the culture medium and quantified metabolite synthesis 24 h later. The quantification of BHB produced over the last day (Day 4 BHB concentration minus Day 3 BHB concentration) shows that the 168FARN-conditioned medium had no effect on BHB secretion by 4T07 cells. In striking contrast, the production of the metabolite by 168FARN more than doubled under the influence of the 4T07-conditioned medium (**Figure 5E**). Thus, the winner cells stimulate the losers to produce a metabolite that boosts the former's proliferation.

## Mechanism of β-Hydroxybutyrate Action

We next asked about the mode of action of BHB on the 4T07 cells. BHB can be imported by four monocarboxylate transporters of the SLC16A gene family, the expression of which varies in different cell types. We assessed the expression of each transporter by RT-QPCR and found that MCT2, MCT3, and MCT4 were barely expressed while MCT1 was highly expressed (**Figure 6A**) in 4T07 cells. This result suggests that MCT1 is likely responsible for the import of BHB in this cell line. Interestingly, we found that MCT1 is three times more expressed in 4T07 than in 168 cells (which, like 4T07, do not express the other MCTs – **Supplementary Figure 4A**), suggesting that the winner cells are more efficient at taking up this metabolite than the losers (**Supplementary Figure 4B**). Finally, incubation of 4T07 with BHB upregulates MCT1, consistent with a positive feedback loop that could increase the transport of this ketone body into the dominant cell line (**Supplementary Figure 4C**).

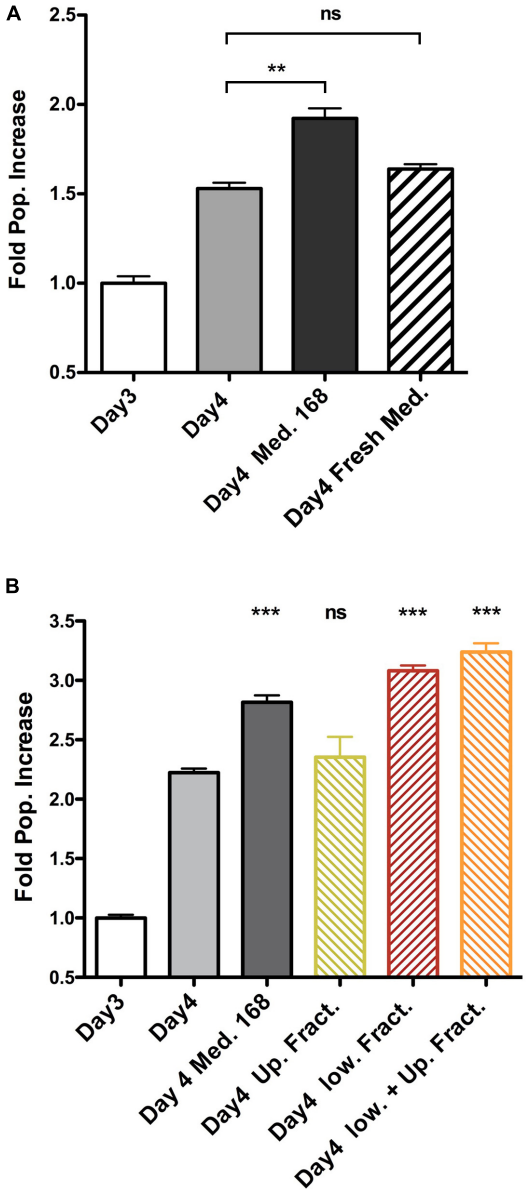


**FIGURE 4 |** Soluble factor secreted by 168FARN cells accelerates proliferation of the 4T07 cells. **(A)** 4T07 cells were grown for 3 days at which point their medium was either left unchanged, or replaced by either 168FARN-conditioned medium or fresh medium, as indicated. Cells were collected 24 h later and counted. Cell numbers at day 3 were arbitrarily set at 1 in order to include the data from three independent experiments. **(B)** The experiment was performed as in panel **(A)**. but the medium conditioned by 168FARN cells was fractionated by membrane ultrafiltration with a 3 KDa molecular cutoff. After complementing the low and the high MW fractions, respectively, with 10% serum and DMEM, the media were used to grow the 4T07 cells, as in panel **(A)**. The two fractions were also combined as a control. ns, not significant; $^{**}p < 0.01$, $^{***}p < 0.001$, all compared to Day 4 point.

β-Hydroxybutyrate can be metabolized and used as a nutrient to replace glucose (Newman and Verdin, 2017). Experiments presented in **Figure 2A** show that fresh medium added at day 3 did not boost cell proliferation, suggesting that in this

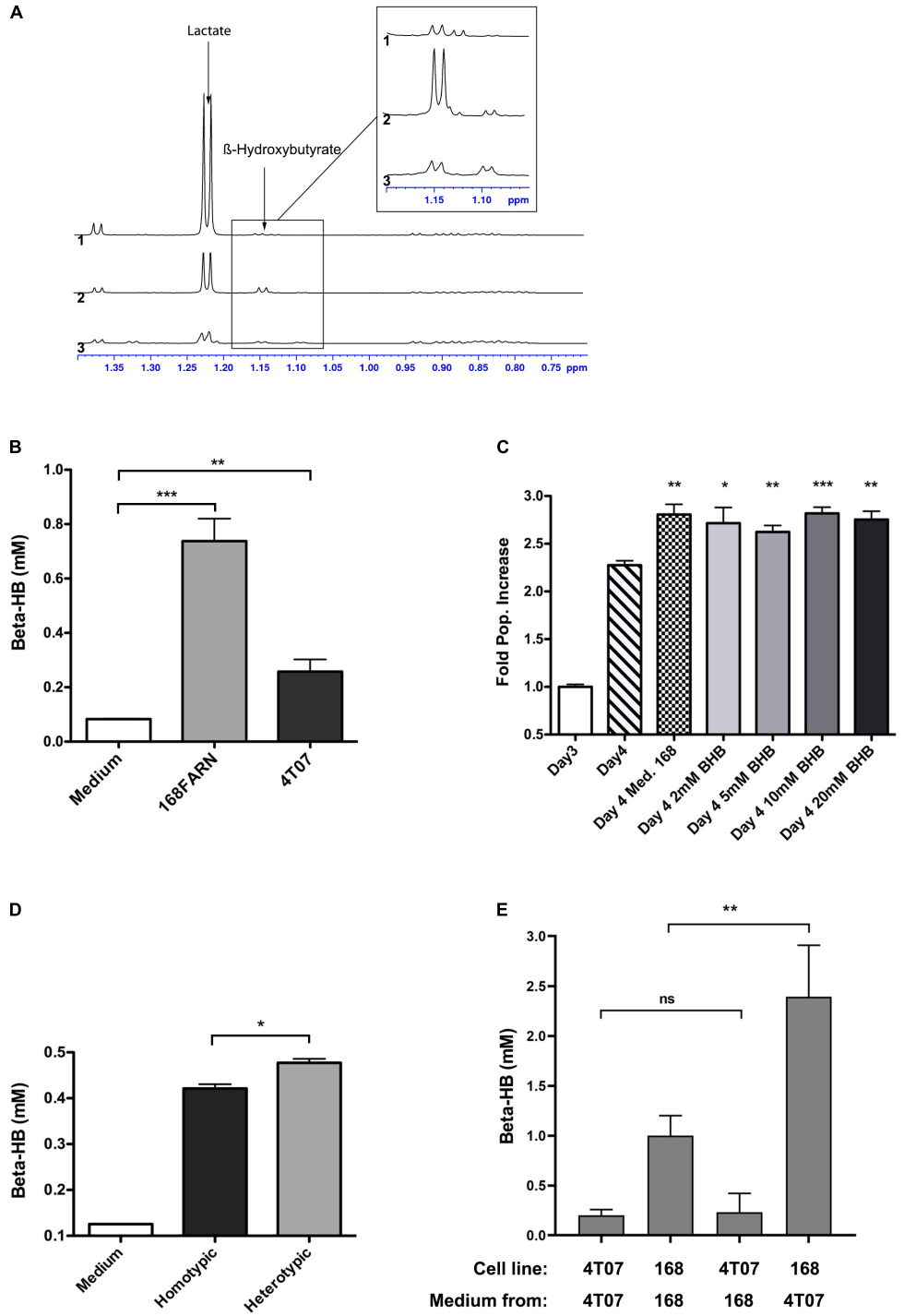


**FIGURE 5 |** Identification of soluble metabolites altering the heterotypic growth dynamics. **(A)** Superimposition of the high-field region of representative 1D proton NMR spectra recorded at 700 MHz, 293 K and pH 7 on samples of culture media collected after growing 40T7 cells (1) or 168FARN cells (2) for 3 days or of fresh cell culture medium (3). The arrows indicate the characteristic resonance of lactate and β-hydroxybutyrate. The insert displays a zoom in this spectral region, revealing the H-alpha resonance of the β-hydroxybutyrate. For all spectra, peak intensities have been normalized on the intensity of the DSS resonance added as internal reference. **(B)** Concentration of β-hydroxybutyrate from fresh medium and from conditioned medium from 168FARN or 4T07 was quantified. **(C)** Commercially available β-hydroxybutyrate at indicated concentrations was added to 4T07 cell culture at day 3 an the growth allowed to proceed for an additional 24 h. All points are compared to Day 4 point. **(D)** 168FARN alone (homotypic) or in 1:1 co-culture with 4T01 cells were grown for 4 days and extracellular β-hydroxybutyrate was measured enzymatically as in **Figure 4B**. **(E)** 168FARN and 4T07 cells were cultured individually for 3 days. The medium was then replaced by the homotypic or heterotypic conditioned one, as indicated, and the culture allowed to continue for an additional 24 h. The β-hydroxybutyrate concentration was quantified at day 4. ns, not significant; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

experimental setup the decrease in the carbon source is not a limiting factor for growth. It is thus unlikely that BHB is used as an energy resource to increase proliferation rate. BHB has previously been identified as an inhibitor of class I histone deacetylases (HDAC) that modulates the expression of genes involved in reactive oxygen species detoxification (Shimazu et al., 2013). Subsequently, another group found that adipocytes use BHB to modulate the expression of a subset of genes involved in the growth of breast cancer cells (Huang et al., 2017). We thus hypothesized that BHB might increase the growth rate of winners through the inhibition of HDACs, thereby modulating the expression of genes involved either in ROS detoxification or in the induction of pro-proliferative factors. In support of this idea, incubation of 4T07 cells either with 168FARN-conditioned medium or with purified BHB increased H3K9 acetylation, albeit to a lesser extent than butyrate, a bona fide HDAC inhibitor (**Figure 6B**).

While we could not detect in 4T07 cells any modification of expression of ROS detoxification genes reported to be regulated by BHB in other cellular models (Shimazu et al., 2013), both BHB and 168-conditioned medium led to significant transcriptional activation of interleukin-11 (IL-11) and lipocalin 2 (LCN2) (**Figure 6C**). Both genes have been previously described to promote cancer cell growth and to be regulated by BHB through its action on HDAC activity (Yang and Moses, 2009; Grivennikov, 2013; Huang et al., 2017). Thus, our data point to the molecular mechanisms involving direct proliferation signaling.

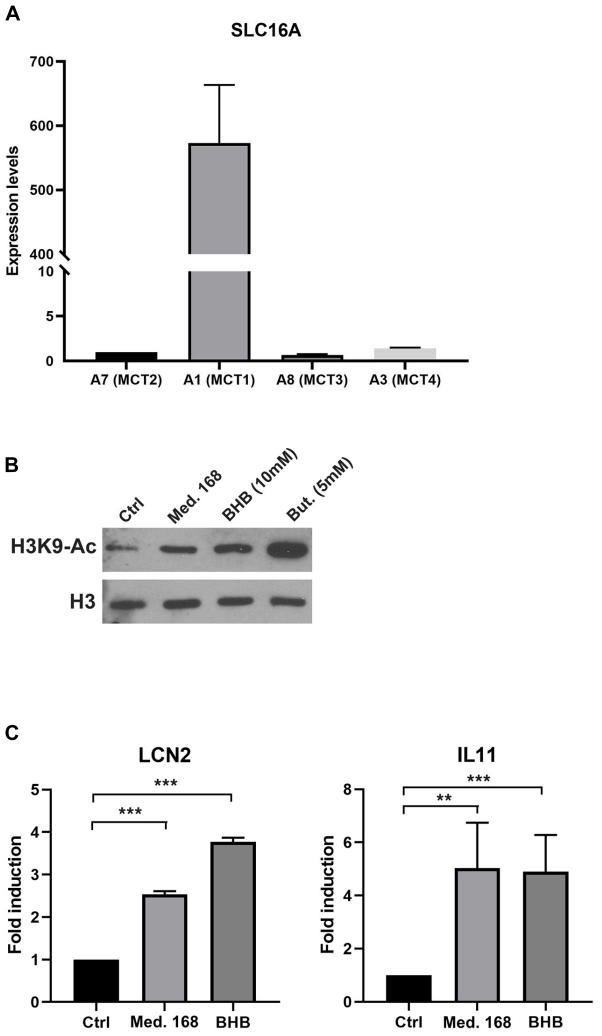


**FIGURE 6 |** Extracellular β-hydroxybutyrate leads to increased H3K9 histone acetylation and altered gene expression in 4T07 cells. **(A)** Expression levels of the slc16A family transporter genes in 4T07 were analyzed by RT-QPCR. Expression of HPRT served as normalization of the data. **(B)** H3K9 histone acetylation was analyzed by immunoblotting of extracts of 4T07 cells grown for 24 h in control, 168-conditioned medium or medium complemented with β-hydroxybutyrate or with butyrate, as indicated. Total histone 3 (H3) abundance served as normalization control. **(C)** 4T07 cells cultured for 3 days were incubated for 8 h with 4T07- (Ctrl) or 168-conditioned medium or purified β-hydroxybutyrate (10 mM) added to fresh medium. Total RNAs were purified and subjected to RT-QPCR with specific primers for LCN2 and IL-11. $^{**}p < 0.01$, $^{***}p < 0.001$.

## Lactate Secretion Slows Down Loser Cell Proliferation

In addition to the positive effect of the 168FARN cells on the proliferation rate of the 4T07 clone, the data shown in **Figure 2** indicate that the latter negatively influences the 168FARN growth dynamics. The NMR analysis highlighted strong lactate production (see **Figure 5A**). This is consistent with our observation of the media color change during culture of the two lines, indicating that the winner clone has a glycolytic type of glucose metabolism leading to a rapid medium acidification in culture. Because extracellular acidification can be detrimental for cell growth, we next asked if 168FARN were particularly sensitive to such growth conditions. We quantified medium acidification by seeding cells at different densities and measuring the extracellular pH after 3 days of culture (**Figure 7A**). As expected, we found that 4T07 cells acidify the medium faster and attain a lower pH during culture compared to 168FARN cells. Indeed, pH ranged from 6.94+/−0.005 (lowest density) to 6.79+/−0.003 (highest density) for the winner line and from 7.38+/−0.008 to 6.92+/−0.006 for 168FARN. To test whether 4T07-mediated extracellular acidification influenced 168FARN growth, we set up a proliferation assay for 168FARN cells grown in medium conditioned by the low and the high density grown 4T07 cells. To control for the effect of pH in the conditioned media, we included a treatment in which the medium from 4T07 was buffered at pH 7.0 by sodium bicarbonate. These experiments revealed that the medium from the low density 4T07 cells (pH 6.94) had no effect on 168FARN proliferation. In contrast, the medium from the high density 4T07 (pH 6.79) drastically decreased the 168FARN growth rate. Moreover, buffering the same medium at pH 7.0 restored the proliferative capacity of 168FARN culture (**Figure 7B**). We conclude that the loser clone is indeed highly sensitive to medium acidification. Taken together our data suggest that the decrease in the growth rate of 168FARN observed in heterotypic conditions is triggered by 4T07 mediated extracellular acidification.

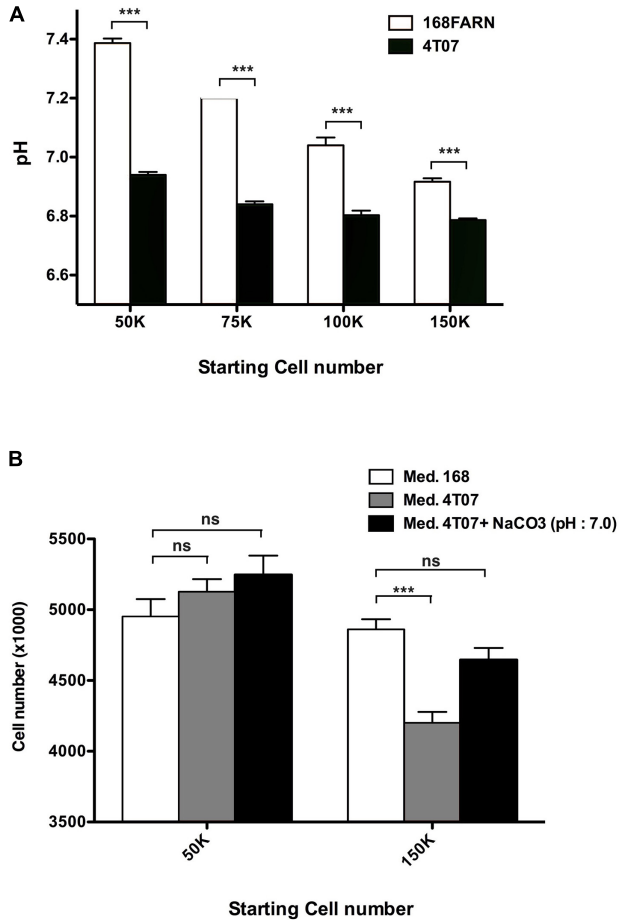


**FIGURE 7 |** Impact of extracellular pH on the loser clone growth. **(A)** 168FARN and 4T07 cells were seeded at the indicated initial densities in six-well plates and cultured for 3 days. Culture media were removed, immediately covered with a layer of mineral oil to prevent oxidation and the pH was measured. **(B)** $10^5$ 168FARN cells were grown for 3 days. Medium was then replaced by conditioned media from cultures grown at low or high density, as indicated. Where indicated, 5 mM $NaCO_3$ was used to buffer the 4T07 conditioned medium to pH 7. Twenty-four hours later cells were harvested and counted. Data are from three independent experiments conducted in triplicates. ns, not significant; ***$p < 0.001$.

## DISCUSSION

Heterogeneity is a ubiquitous feature of tumors that influences growth and metastasis, and thus the potential for therapeutic success. Ecological interactions between subclones are key to the emergence of this heterogeneity, yet only few empirical studies have characterized the nature of these interactions or their underlying mechanisms. These include commensal (Kaznatcheev et al., 2019; Farrokhian et al., 2020) and cooperative (Cleary et al., 2014) interactions *in vitro*, and how such interactions can drive tumor invasion (Chapman et al., 2014) and metastasis *in vivo* (Janiszewska et al., 2019; Naffar-Abu Amara et al., 2020).

Our study extends previous work (Robinson and Jordan, 1989; Marusyk et al., 2014; Archetti et al., 2015) by demonstrating that two cell lines derived from the same tumor exhibit a sophisticated relationship, whereby one (the "winner") effectively farms the population of the other (the "loser"). We further identified key metabolites (BHB and lactate) that regulate these interactions between the winning and losing clones. Similar to Archetti et al. (2015), we found that exploitative clonal interactions evolve through time, but whereas these authors observed a frequency-dependent change that could explain clonal coexistence, we were unable to detect this effect. Simple mathematical analysis within the framework of evolutionary game theory nevertheless shows that, when accounting for microenvironmental heterogeneity, our inferred parameter values are plausibly consistent with long-term clonal coexistence (see section "Materials and Methods").

Because our *in vitro* experiments simplify the diverse, complex interrelationships that predominate in spatially complex microenvironments, the parameter values we have inferred may not precisely translate to *in vivo* contexts. For example, the scenario of our experimental model, which depends on microenvironmental acidification by the winner clone, may be less relevant to micrometastases that are small enough to maintain physiological pH (De Palma et al., 2017; Beckman et al., 2020). On the other hand, there is an overwhelming consensus that in larger tumors (both primary and metastatic), neoangiogenesis produces abnormal, leaky vessels that give rise to poor oxygenation and acidic conditions (De Bock et al., 2011), consistent with our experimental system. That paracrine signaling is responsible for the effects we observed between winner and loser cell lines suggests that the spatial arrangement of these cells could be crucial to their growth and relative frequencies *in situ* (Archetti et al., 2015). The effect of spatial structure would depend on the typical distance that secreted molecules travel through the complex tumor microenvironment. Our results indicate that areas of contact or close proximity between the two subclones will grow faster and therefore come to dominate spatially isolated populations, producing what is effectively a mixed 4T07–168FARN "phenotype." The actual spatial arrangement of these two subclones in the original tumor is unknown, but the authors of the study originally isolating these cell lines note that they may represent only a small sample of the tumor's diversity (Dexter et al., 1978). A growing body of evidence suggests that single, site-specific biopsies may be of little use in quantifying spatial heterogeneity, due to the multiscale (local, regional, metastatic) nature of tumor evolution (Amirouchene-Angelozzi et al., 2017). Computational modeling indicates that the range of cell–cell interaction and the mode of cell dispersal are crucial factors determining the pattern of intratumor heterogeneity and associated characteristics of tumor growth and evolutionary potential (Waclaw et al., 2015; Noble et al., 2020). While a comprehensive description of intra-tumoral ecological interactions is a daunting task, beyond the power of existing technology, a fuller understanding of their general features is essential for devising therapies aimed at rendering cancer a chronic, controllable disease (Gatenby and Brown, 2020; Viossat and Noble, 2021).

We find that the complex interactions between the 4T07 and 168FARN cells are governed by paracrine signaling emanating from both clones. This mechanistic conclusion differs from the

original observations reported by Miller et al. (1988). Indeed, in the original publication the results concerning the inhibitory effect of 4T07 conditioned media on 168 cells were inconclusive. This apparent discrepancy could be due to slightly different culture conditions used in the two sets of experiments. Indeed, the medium acidification due to the lactate release by the 4T07 that is responsible for slowing down the growth of 168 cells reaches the required threshold value only after prolonged culture (3–4 days under our experimental conditions). It is thus possible that in the original report the culture time and/or the cell density were insufficient for the clear visualization of the paracrine effect of the winners on the losers. Moreover, Miller et al. (1988) did not investigate the paracrine effect exerted by the 168 on the 4T07 cells. Our results are the first to show the reciprocal effects of both cell lines on each other, thus highlighting the complexity of their mutual interactions.

We have identified a ketone body, BHB, which is produced by loser cells and acts to increase the growth rate of winner cells. Mechanistically, the competitive advantage afforded by BHB to the winner clone appears to be mediated through the HDAC-controlled activation of a genetic program that boosts its proliferation. Ketone bodies are small lipid-derived molecules, physiologically produced by the liver and distributed via the circulation to metabolically active tissues, such as muscle or brain (Newman and Verdin, 2017), where they serve as a glucose-sparing energy source in times of fasting or prolonged exercise. Recently, several studies reported that cell types such as adipocytes, intestinal stem cells or cancer cells originating from colorectal carcinoma or melanoma can also produce BHB (Grabacka et al., 2016; Huang et al., 2017; Shakery et al., 2018; Cheng et al., 2019). Our results identifying BHB as a signaling molecule involved in intra-tumoral clonal interactions fall into the general category of these novel roles for ketone bodies in cell communication.

However, the link between ketone bodies and tumor development remains controversial. On the one hand, it was shown that ketonic diet slows down tumor development in brain cancer mice models (Poff et al., 2013, 2014). On the other hand, our results together with other recent data (Huang et al., 2017) suggest that BHB may favor breast cancer progression. One unexplored possibility to explain these contradictory observations is that this ketone body can be used differently by different cancer cell types, for example as a carbohydrate supply or as a HDAC inhibitor, ultimately leading to cancer-type and context specific response.

In our experimental model, BHB increases winner cells proliferation by activating a genetic program through HDAC inhibition. Among the genes we discovered to be activated by the ketone body, IL-11 is an interleukin that displays a pro-proliferative activity (Grivennikov, 2013). Interestingly, in a distinct breast cancer cell cooperation model, sub-clonal expression of IL-11 favors the expansion not only of cells that express it, but also of other cellular sub-clones (Marusyk et al., 2014). This suggests that IL-11 acting in either paracrine or autocrine fashion could lead, respectively, to cooperation or to competition between subclones, thus participating actively in the selection and evolution of tumor heterogeneity.

Overall, our experimental data therefore suggest a model in which the winner line stimulates the production of and benefits from a compound delivered by the loser line and, conversely, the loser is negatively influenced by the presence of winners through secretion of another compound.

We note that while in artificially maintained conditions of non-constrained growth (in culture) the losers are eventually eliminated, many additional selective pressures that may affect clonal fitness operate *in vivo*. These involve cellular response to physical cues due to crowding (Vishwakarma and Piddini, 2020) and interactions with the extracellular matrix (Lu et al., 2012) as well as response to signaling from the stroma, including its inflammatory and immune components (Quail and Joyce, 2013). These elements are expected to influence the outcome of the direct interactions between the tumoral clones and may change the nature of their ecological interaction from net exploitation (*in vitro*) to mutual benefit (*in vivo*). Future study should evaluate whether parasitic effects are observed *in vivo*, and determine the extent to which these cell–cell interactions mediate important tumor characteristics, including growth, drug resistance, and metastatic behavior.

# MATERIALS AND METHODS

## Cell Culture

4T07 and 168FARN were a kind gift of Robert Hipskind. All cell lines were cultured in Dulbecco's modified Eagle medium containing 10% fetal bovine serum, 100 ng/mL streptomycin, and 100 U/mL penicillin at 37°C with 5% CO2.

For co-culture experiments a mixture of GFP-labeled and parental cells (empty-vector transduced) cells were seeded at the final density of $10^5$ cells/well in six-well plates, except where mentioned otherwise. Upon reaching confluence (3–4 days) they were harvested, diluted to the original density and replated. The remaining fraction was analyzed by flow cytometry.

## Immunoblot Analysis

Cells were lysed in boiling Laemmli buffer supplemented with protease inhibitors, then sonicated and complemented with DTT. Protein concentration was determined by BCA (Thermo Scientific) assay. Fifteen to twenty micrograms of total protein were loaded onto SDS-PAGE gels and transferred onto nitrocellulose membranes. The membrane was blocked with TBST (1× TBS with 0.1% Tween 20) + 5% milk at room temperature for 1 h and incubated with primary antibody and then with horseradish peroxidase (HRP)-coupled secondary antibody (Amersham, Piscataway, NJ, United States). Activity was visualized by electrochemiluminescence. Antibodies used in this study are anti-Histone H3 (Cell signaling Technology #9717) and anti-Acetyl-Histone H3 (Lys9) (Cell signaling Technology #9649).

## Reverse Transcription and Real-Time PCR

Total mRNA was isolated using a RNeasy mini kit (Qiagen, Germantown, MD, United States). Reverse transcription

was performed with random hexamers and M-MLV Reverse Transcriptase (Invitrogen). Real-time PCR was performed in triplicates with LC FastStart DNA Master SYBR Green I on a LightCycler rapid thermal cycler system (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. Housekeeping gene HPRT was used for normalization. Primers sequences are available upon request.

## Time-Lapse Microscopy

Time-lapse microscopy was performed at 37°C with 5% CO2, with images taken at 45-min intervals using an inverted Zeiss Axio-Observer microscope. The images were processed and analyzed using ImageJ software.

## EdU Staining

Cells were incubated with 10 μM EdU for 2 h, harvested and processed using the Click-iT$^{TM}$ EdU Alexa Fluor$^{TM}$ 647 Flow Cytometry Assay Kit (Thermo Fisher Scientific #C10424) following manufacturer instructions. Labeled cells were then analyzed on a FACSCalibur flow cytometer using CellQuest Pro software (BD Biosciences).

## Apoptosis Quantification

To determine the percentage of apoptotic cells with externalized phosphatidylserine (PS), adherent and floating cells were collected and labeled with the Annexin V-Cy3 Apoptosis Detection Kit (Abcam, Cambridge, United Kingdom, #ab14143) according to the manufacturer's instructions. Labeled cells were then analyzed on a FACSCalibur flow cytometer using CellQuest Pro software (BD Biosciences).

## β-Hydroxybutyrate Quantification

β-Hydroxybutyrate concentration was measured by an enzymatic kit (Sigma-Aldrich MAK041) following the manufacturer instructions. Briefly, β-hydroxybutyrate present in the culture medium was determined by a coupled enzyme reaction, resulting in a colorimetric (450 nm) product, proportional to the β-hydroxybutyrate concentration. The absorbance was measured on a spectrophotometer.

## Medium Fractionation

In order to separate low molecular weight molecules from the conditioned culture medium, 5–10 ml were loaded on a Vivaspin Turbo 15 PES, 3,000 MWCO column (Sartorius VS15T91) and centrifuged at 4000 × $g$ for 30 min following the manufacturer instructions. Both fractions were then used for subsequent experiments and RMN analysis.

## RMN Analysis

NMR experiments were recorded at 293 K and pH 7 on an AVANCE III BRUKER spectrometer operating at 700 MHz (proton frequency), using a Z-gradient shielded TCI 1H-13C-15N cryoprobe. Fully relaxed 1D 1H spectra were aquired with the regular 1D NOESY, using 5 s as relaxation delay. The samples consisted on 1.5 mL of cell media (fresh or conditioned by cell culture), lyophilized and dissolved in 500 μL of deuterated phosphate buffer (50 mM, pH 7). DSS (EURISOTOP©, final concentration: 0.5 mM) was added as internal reference for chemical shift referencing and as a concentration standard for spectra normalization. The assignment of the 1H resonances of the compound of interest in this study (lactate, β-hydroxybutyrate) was based on chemical shifts reported on the literature (1) and further confirmed using 2D [1H, 1H] (TOCSY) and [1H-13C] (HSQC, HSQC-TOCSY) NMR spectroscopy.

## Statistical Analysis

Experiments were repeated at least three times. Data are presented as mean ± SEM. An Independent Student's $t$-test was performed to analyze the assay results; a two-tailed Student's $t$-test was used to compare the intergroup differences. Significance was accepted for values where $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***).

## Overview of Mathematical Methods

Our aim is to determine the general nature of the evolutionary dynamics in a form that can be readily compared to other systems (as opposed to generating quantitative predictions for our particular system). Accordingly, we chose to fit a simple, standard model to each distinct phase of the dynamics, such that the inferred parameter values have straightforward ecological interpretations. A key advantage of our method is that it is generic; in principle, the same method can be applied to any experimental evolution set-up with two competing populations of cancer cells, bacteria, or other entities.

This mathematical approach is in the same vein as that of Kaznatcheev (2017) and Kaznatcheev et al. (2019) but with three important differences. First, our method can accommodate a smaller data set and is thus more economical because we mostly rely on measurements of initial and final proportions in competition assays, such as can be determined via flow cytometry, rather than extensive time-lapse image analysis. Second, whereas Kaznatcheev (2017) and Kaznatcheev et al. (2019) confine their analysis to exponential or logistic growth phases, we also examine phases in which cell populations exhibit non-logistic dynamics. Third, because we consider non-logistic growth phases, we use a density-dependent rather than a frequency-dependent model.

We note that to make quantitative predictions of outcomes in different scenarios, we would require a different type of model with equations describing the dynamics of paracrine factors mediating clonal interactions. This more complicated model would include several more parameters and design choices (for example, how each paracrine factor's production rate and its effects vary with its concentration) and would thus be non-identifiable in the absence of detailed paracrine concentration measurements. Obtaining such measurements remains as a challenge for future studies.

## Definitions and Mathematical Relationships

We define the intrinsic growth rate as the exponential growth rate in the absence of interactions. In the Lotka–Volterra differential

equations, this parameter is multiplied by the population size of the respective type. The intrinsic growth rate is the limit of the net growth rate as the population sizes approach zero (when interaction terms are negligible).

We define the net growth rate as the actual rate of change of the population size (i.e., the time derivative), which is the sum of the basic growth rate and interaction terms.

**Supplementary Figures 6, 7** illustrate some of the mathematical relationships relevant to our methods.

## Dynamical Models and Inference From Homotypic Growth Curves

We describe the exponential phase 1 dynamics as

$$\frac{dL}{dt} = Lr_{L,1},\ \frac{dW}{dt} = Wr_{W,1},$$

where $L$ (loser) and $W$ (winner) are the population sizes of 168 and 4T07, respectively, and $r_{L,1}$ and $r_{W,1}$ are the respective growth rates.

In phase 2, we assume a density-dependent competitive Lotka–Volterra model, parameterized in terms of intrinsic growth rates $r_{L,2}$ and $r_{W,2}$ and interaction terms $a$, $b$, $c$ and $d$:

$$\frac{dL}{dt} = L(r_{L,2} + aL + bW),\frac{dW}{dt} = W(r_{W,2} + cL + dW).$$

In the homotypic case, terms $bW$ and $cL$ vanish and the phase 2 model is equivalent to logistic growth. We combine the two models and fit to the normalized time-lapse data for the homotypic growth curves using least-squares with R package deSolve (Soetaert et al., 2010) to infer the values of $r_{L,1}$, $r_{W,1}$, $r_{L,2}$, $r_{W,2}$, $a$, and $d$.

In phase 3, we assume the same model as in phase 2 except we replace $r_{W,2}$ by $r_{W,3}$ to account for the change in the 4T07 net growth rate (equivalent to adding a density-dependent death rate).

## Inferring Between-Type Interaction Terms

To infer the interaction parameters $b$ and $c$ we need data that covers a wide range of proportions of the two cell types. Since our time-lapse data is limited to only a few initial conditions, we fit the model to the outcomes of serial competition assays, and we employ the heterotypic time-lapse data for validation only. First we define

$$l = \frac{L}{W+L},\ w = \frac{W}{W+L},$$
$$s = \log\frac{w}{l} = \log\frac{w}{1-w} = \text{logit}(w).$$

The time derivative of the $s$ is then equal to the net growth rate difference, which in phase 2 is

$$\frac{ds}{dt} = r_{W,2}-r_{L,2} + (d-b)W + (c-a)L.$$

In the limit $w \to 1$, the final term $(c-a)L$ is negligible and we can obtain $b$ in terms of $\frac{ds}{dt}$, $W$, and parameters whose values we have already inferred, as follows:

$$\frac{ds}{dt} = r_{W,2}-r_{L,2} + (d-b)W \Rightarrow b = \frac{\frac{ds}{dt}-r_{W,2}+r_{L,2}}{W} + d.$$

To obtain $W$, we note that in the limit $w \to 1$,

$$\frac{dW}{dt} = W(r_{W,2} + dW),$$

which is the logistic differential equation with solution

$$W(t) = \frac{W(t_1)re^{rt}}{r - W(t_1)(e^{rt}-1)d},$$

where $r = r_{W,2}$ and $t_1$ is the time at which phase 2 begins. We can thus use our previously inferred parameter values to obtain $W(t)$ at every time $t$ in phase 2 (note that if there were not an analytical solution then we could have solved the equation numerically).

Since $W$ and $\frac{ds}{dt}$ are linearly related, we can replace them by their mean values:

$$\frac{\text{mean}\left(\frac{ds}{dt}\right)-r_{W,2}+r_{L,2}}{\text{mean}(W)} + d$$
$$= \frac{\text{mean}\left(\frac{ds}{dt}\right)-r_{W,2}+r_{L,2}}{\text{mean}\left(\frac{\frac{ds}{dt}-r_{W,2}+r_{L,2}}{b-d}\right)} + d = b.$$

Using the mean values to calculate $b$ is convenient as our competition assays reveal only the initial and final values of $s$. Specifically, we take the means in the interval $[t_1, t_2]$, where $t_2$ is the time at which phase 2 ends and

$$\text{mean}\left(\frac{ds}{dt}\right) = \frac{s(t_2)-s(t_1)}{t_2-t_1} = \frac{\triangle s}{\triangle t}.$$

It remains only to obtain the value of the above expression – known as the gain function – in the limit $w(t_1) \to 1$. From competition assay data, we can immediately obtain $s(t_2) = \log\frac{w(t_2)}{1-w(t_2)}$ for each value of $s(0) = \log\frac{w(0)}{1-w(0)}$. To infer $w(t_1)$ and $s(t_1)$, we need to adjust for the exponential growth of both cell types during phase 1:

$$s(t_1) = s(0) + t_1(r_{W,2}-r_{L,2})$$
$$\Longrightarrow \text{logit}(w(t_1)) = \text{logit}(w(0)) + t_1(r_{W,2}-r_{L,2})$$
$$\Longrightarrow w(t_1) = \text{logit}^{-1}\left(\text{logit}(w(0)) + t_1\left(r_{W,2}-r_{L,2}\right)\right).$$

We thus obtain the values of $s(t_1)$ and $w(t_1)$ in each competition assay. Finally, we determine by linear regression the relationship between $\triangle s/\triangle t$ and $w(t_1)$ (**Supplementary figure 5B**) and, from the equation of the regression line, infer the value of $\triangle s/\triangle t$ in the limit $w(t_1) \to 1$. We then have everything required to infer the value of $b$. By an analogous method (switching $L$ and $W$, $b$ and $c$, and $a$ and $d$) we also infer the value of $c$.

## Excluding Results of First-Round Competition Assays

In our regression to determine the relationship between $\triangle s/\triangle t$ and $w(t_1)$, we excluded data from the first round of competition assays (days 0–3 in **Figures 2B,C**) because these measurements were unusually variable, and this variance was most likely an experimental artifact. Specifically, setting up the initial experiment took substantially longer than carrying out subsequent replatings as additional steps were required before seeding the cells. Since cells were kept for longer in suspension before the first round, they will have experienced more stress and potentially mortality. This means that results of the first round of competition assays are likely to be less reliable than results of subsequent rounds. For completeness, **Supplementary Figures 5C,D** show linear regression applied to the entire data set, including the first round.

## Carrying Capacities

To find carrying capacities, we note that the phase 2 model can alternatively be parameterized as

$$\frac{dL}{dt} = Lr_{L,2}\left(1-\frac{L + \beta W}{K_L}\right), \frac{dW}{dt} = Wr_{W,2}\left(1-\frac{\gamma L + W}{K_W}\right),$$

where the parameters are calculated as in **Table 1**. The carrying capacities $K_W$ and $K_L$ are the upper limits approached by the population sizes of $W$ and $L$, respectively, during phase 2.

## Potential for Coexistence *in vivo*

In a growing tumor, we expect cell–cell competition to be less than in our *in vitro* experiments, because, in the former, resources are continually replenished and waste materials removed by the host circulatory system. The evolutionary dynamics will then mostly depend on the difference in intrinsic growth rates and interactions mediated by diffusible factors. Furthermore, during tumor growth, the dynamics may be better described by a frequency- rather than a density-dependent model. We can then describe the evolutionary dynamics within the framework of evolutionary game theory using the payoff matrix

$$\begin{pmatrix} \beta_L-\gamma & \alpha_L-\gamma \\ \beta_W & \alpha_W \end{pmatrix},$$

where $\alpha_L, \alpha_W < 0$ denote the harm inflicted by $W$ on $L$ and $W$, respectively; $\beta_L, \beta_W > 0$ are the benefits bestowed by $L$ to $L$ and $W$, respectively; and $\gamma > 0$ is the difference between the intrinsic exponential growth rates. The relative values of the entries in the payoff matrix determine which game (for example, prisoner's dilemma or hawk–dove) is equivalent to the evolutionary dynamics.

The parameter values inferred for phase 2 of the competition assays imply

$$\beta_W > \beta_L-\gamma > \alpha_W > \alpha_L-\gamma,$$

in which case the evolutionary dynamics are equivalent to a prisoner's dilemma game for which $W$ is the only evolutionarily stable strategy (ESS). This means that $W$ (4T07) can invade and stably replace a population of $L$ (168).

If instead $\alpha_L-\gamma > \alpha_W$ then the payoff matrix defines a hawk–dove game that permits coexistence. In this scenario, $W$ harms itself more than it harms $L$, and this difference outweighs $W'$s higher intrinsic growth rate. This could happen, for example, if harmful factors produced by $W$ imperfectly diffuse, so that $W$ cells experience a higher concentration than $L$ cells. At the mixed ESS, the $W$ proportion is

$$\frac{\alpha_W-\alpha_L + \gamma}{\alpha_W-\alpha_L + \beta_L-\beta_W}.$$

However, if additionally $\beta_L-\beta_W > \gamma$ (so that $L$ benefits itself more than it benefits $W$, and this difference outweighs $W'$s higher intrinsic growth rate) then coexistence again becomes impossible as the game again becomes a prisoner's dilemma but with $L$ as the ESS.

In a resource-poor environment, we might describe the evolutionary dynamics using the payoff matrix

$$\begin{pmatrix} \beta_L-\gamma & \alpha_L-\gamma \\ \beta_W-\delta & \alpha_W-\delta \end{pmatrix},$$

where $\delta$ is the reduction in $W'$s intrinsic growth rate due to the degraded environment (as inferred for phase 3 of our 96-h competition assays). This scenario favors $L$ and suggests that $L$ may be the ESS in a resource-poor environment, such as hypoxic regions within a tumor.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

UH, MH, and PL conceived the study. VW, CR, and PL designed and performed the experiments and analyzed the data. RN designed and performed the mathematical modeling and mathematical analysis. RN, UH, MH, and PL wrote the manuscript with contributions from VW and CR. All authors contributed to the article and approved the submitted version.

## FUNDING

The authors wish to thank HTE "HetCoLi" (HTE20161) and ITMO "Physique Cancer" (CanEvolve PC201306) for funding. RN acknowledges support from the National Cancer Institute of the National Institutes of Health under Award Number U54CA217376. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. CR was supported by the French Infrastructure for Integrated Structural Biology (FRISBI) (grant no. ANR-10-INSB-05). MH thanks the McDonnell Foundation for funding (Studying Complex Systems research award 220020294).

## ACKNOWLEDGMENTS

We are grateful to Emie Quissac and Yasser Kerboua for their excellent technical assistance, and to Artem Kaznatcheev for helpful conversations.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.675638/full#supplementary-material

**Supplementary Figure 1 | (A,B)** Growth dynamics of subclones under homotypic and heterotypic conditions. $10^5$ Cells were seeded at a 3:1 **(A)** or 1:4 **(B)** ratios in homotypic (parental and GFP expressing derivative of the same cell line) or heterotypic (different cell lines, one expressing GFP) co-cultures and harvested and replated at the initial densities ($10^5$ cells/plate) at indicated times. The ratios of GFP-labeled to unlabeled cells were estimated by flow cytometry. The results represent data from three independent experiments and are shown as mean ± SEM.

**Supplementary Figure 2 | (A)** Apoptosis quantification of subclones under homotypic and heterotypic conditions. A total of $10^5$ cells were seeded. 168G cells were co-cultured with either the 168P (homotypic) or 4T07P (heterotypic) cells at a 1:1 ratio for 4 days and harvested. Apoptosis was quantified by flow cytometry following Annexin-V staining. ns: not significant. **(B)** S phase quantification of subclones under homotypic and heterotypic conditions. A total of $10^5$ cells were seeded. 168G cells were co-cultured with either the 168P (homotypic) or 4T07P (heterotypic) cells at a 1:1 ratio for 4 days. Before harvesting at day 4 cells were labeled by a 2 h pulse of EdU and the fraction of cells in the S phase was determined by flow cytometry. $^*p < 0.05$, $^{**}p < 0.01$.

**Supplementary Figure 3 |** Growth dynamics of subclones at low and high density. Experiments were performed as in **Figure 3B**. Cells were grown in heterotypic conditions at a starting ratio of 1:1. Cells were seeded either at low density (50k) or high density (150k), diluted, and quantified every 3 days. At low density, cells do not reach confluence before replating. The results represent data from three independent experiments and are shown as mean ± SEM.

**Supplementary Figure 4 | (A)** Expression levels of the slc16A family transporter genes in 168FARN. RT-QPCR analysis was performed on 168FARN RNA for Mct2, Mct1, Mct3, and Mct4 genes and normalized to HPRT. Relative expression levels were compared to Mct2. **(B)** Slc16A1 expression in both subclones. Slc16A1 RNA levels were monitored by RT-QPCR, normalized with HPRT and adjusted relative to levels in 168FARN cells. **(C)** Influence of Slc16A1 expression by β-hydroxybutyrate. Experiment was performed as in **Figure 5B**. Slc16A1 RNA levels were quantified as in panel **(A)** and adjusted relative to levels in control condition. $^{***}p < 0.001$.

**Supplementary Figure 5 | (A)** Mean net growth rate difference (gain function) versus initial 4T07 proportion in phases 1 and 2 (purple) and phases 1, 2, and 3 (green). Each point corresponds to the outcome of a competition assay. Regression lines are shown with 95% confidence intervals. **(B)** Mean net growth rate difference versus initial 4T07 proportion in phase 2 (purple) and phases 2 and 3 (green). This data set was obtained from the data shown in panel **(A)** by adjusting for exponential growth in phase 1 (see section "Materials and Methods"). **(C)** The same as panel **(A)** but including results for the first round of competition assays (days 0–3). First-round measurements were excluded from analyses as they were unusually variable and unreliable due to an experimental artifact (see section "Materials and Methods"). **(D)** The same as panel **(B)** but including results for the first round of competition assays (days 0–3).

**Supplementary Figure 6 |** Relationship between population dynamics and net growth rates. The net growth rate of each cell type (right column) is the derivative of its log-transformed growth curve (left column). **(A)** Mathematical model dynamics. From the dynamical model, net growth rates can be found precisely by evaluating differential equation terms. The model was parameterized with values inferred from data (**Table 1**) and initiated with a 3:1 ratio of 168–4T07. **(B)** Empirical dynamics. From time-lapse data, net growth rates can be approximated as local gradients (difference quotients). In this example, we estimated net growth rates from smoothed growth curves by calculating difference quotients across a 5-h span. Smoothed growth curves (not shown) were obtained by computing running medians with a 5-h span. Since we did not use heterotypic time-lapse data for parameter inference, the resemblance between the two rows of this figure contributes to validating our model. The data in panel **(B)** is the same as in **Figures 3A,B**.

**Supplementary Figure 7 |** Mathematical relationships relevant to our methods. The diagram illustrates several equivalent ways of calculating the mean growth rate difference (gain function, blue) from the parameterized dynamical model (red). Also shown is our method of calculating the gain function from competition assay data (orange).

## REFERENCES

Amirouchene-Angelozzi, N., Swanton, C., and Bardelli, A. (2017). Tumor evolution as a therapeutic target. *Cancer Discov.* 7, 805–817. doi: 10.1158/2159-8290.cd-17-0343

Archetti, M., Ferraro, D. A., and Christofori, G. (2015). Heterogeneity for IGF-II production maintained by public goods dynamics in neuroendocrine pancreatic cancer. *Proc. Natl. Acad. Sci. USA* 112, 1833–1838. doi: 10.1073/pnas.1414653112

Aslakson, C. J., Rak, J. W., Miller, B. E., and Miller, F. R. (1991). Differential influence of organ site on three subpopulations of a single mouse mammary tumor at two distinct steps in metastasis. *Internat. J. Cancer* 47, 466–472. doi: 10.1002/ijc.2910470327

Axelrod, R., Axelrod, D. E., and Pienta, K. J. (2006). Evolution of cooperation among tumor cells. *Proc. Natl. Acad. Sci. USA* 103, 13474–13479. doi: 10.1073/pnas.0606053103

Beckman, R. A., Kareva, I., and Adler, F. R. (2020). How Should Cancer Models Be Constructed? *Cancer Control* 27, 1–12.

Calbo, J., van Montfort, E., Proost, N., van Drunen, E., Beverloo, H. B., Meuwissen, R., et al. (2011). A functional role for tumor cell heterogeneity in a mouse model of small cell lung cancer. *Cancer Cell* 19, 244–256. doi: 10.1016/j.ccr.2010.12.021

Chapman, A., del Ama, L. F., Ferguson, J., Kamarashev, J., Wellbrock, C., and Hurlstone, A. (2014). Heterogeneous tumor subpopulations cooperate to drive invasion. *Cell Rep.* 8, 688–695. doi: 10.1016/j.celrep.2014.06.045

Cheng, C.-W., Biton, M., Haber, A. L., Gunduz, N., Eng, G., Gaynor, L. T., et al. (2019). Ketone Body Signaling Mediates Intestinal Stem Cell Homeostasis and Adaptation to Diet. *Cell* 178, 1115.e–1131.e.

Cleary, A. S., Leonard, T. L., Gestl, S. A., and Gunther, E. J. (2014). Tumour cell heterogeneity maintained by cooperating subclones in Wnt-driven mammary cancers. *Nature* 508, 113–117. doi: 10.1038/nature13187

De Bock, K., Cauwenberghs, S., and Carmeliet, P. (2011). Vessel abnormalization: another hallmark of cancer?: Molecular mechanisms and therapeutic implications. *Curr. Opinion Genet. Dev.* 21, 73–79. doi: 10.1016/j.gde.2010.10.008

De Palma, M., Biziato, D., and Petrova, T. V. (2017). Microenvironmental regulation of tumour angiogenesis. *Nat. Rev. Cancer.* 17, 457–474. doi: 10.1038/nrc.2017.51

Dexter, D. L., Kowalski, H. M., Blazar, B. A., Fligiel, Z., Vogel, R., and Heppner, G. H. (1978). Heterogeneity of tumor cells from a single mouse mammary tumor. *Cancer Res.* 38, 3174–3181.

Farrokhian, N., Maltas, J., Ellsworth, P., Durmaz, A., Dinh, M., Hitomi, M., et al. (2020). Dose dependent evolutionary game dynamics modulate competitive release in cancer therapy. *bioRxiv* 2020:18.303966.

Gatenby, R. A., and Brown, J. (2017). Mutations, evolution and the central role of a self-defined fitness function in the initiation and progression of cancer. *Biochim. Biophy.Acta* 1867, 162–166. doi: 10.1016/j.bbcan.2017.03.005

Gatenby, R. A., and Brown, J. (2020). Integrating evolutionary dynamics into cancer therapy. *Nat. Rev. Clin. Oncol.* 17, 675–686. doi: 10.1038/s41571-020-0411-1

Grabacka, M. M., Wilk, A., Antonczyk, A., Banks, P., Walczyk-Tytko, E., Dean, M., et al. (2016). Fenofibrate Induces Ketone Body Production in Melanoma and Glioblastoma Cells. *Front. Endocrinol.* 2016:7. doi: 10.3389/fendo.2016.00005

Greaves, M., and Maley, C. C. (2012). Clonal evolution in cancer. *Nature* 481, 306–313.

Grivennikov, S. I. (2013). IL-11: a prominent pro-tumorigenic member of the IL-6 family. *Cancer Cell* 24, 145–147. doi: 10.1016/j.ccr.2013.07.018

Henke, J., Willker, W., Engelmann, J., and Leibfritz, D. (1996). Combined extraction techniques of tumour cells and lipid/phospholipid assignment by two dimensional NMR spectroscopy. *Anticancer Res.* 16, 1417–1427.

Heppner, G. H. (1984). Tumor heterogeneity. *Cancer Res.* 44, 2259–2265.

Huang, C.-K., Chang, P.-H., Kuo, W.-H., Chen, C.-L., Jeng, Y.-M., Chang, K.-J., et al. (2017). Adipocytes promote malignant growth of breast tumours with monocarboxylate transporter 2 expression via β-hydroxybutyrate. *Nat. Comm.* 8:14706.

Janiszewska, M., Tabassum, D. P., Castaño, Z., Cristea, S., Yamamoto, K. N., Kingston, N. L., et al. (2019). Subclonal cooperation drives metastasis by modulating local and systemic immune microenvironments. *Nat. Cell Biol.* 21, 879–888. doi: 10.1038/s41556-019-0346-x

Kaznatcheev, A. (2017). Two conceptions of evolutionary games: reductive vs effective. *bioRxiv* 2017:231993.

Kaznatcheev, A., Peacock, J., Basanta, D., Marusyk, A., and Scott, J. G. (2019). Fibroblasts and alectinib switch the evolutionary games played by non-small cell lung cancer. *Nat. Ecol. Evol.* 3, 450–456. doi: 10.1038/s41559-018-0768-z

Lu, P., Weaver, V. M., and Werb, Z. (2012). The extracellular matrix: A dynamic niche in cancer progression. *J. Cell Biol.* 196, 395–406. doi: 10.1083/jcb.201102147

Maley, C. C., Aktipis, A., Graham, T. A., Sottoriva, A., Boddy, A. M., Janiszewska, M., et al. (2017). Classifying the evolutionary and ecological features of neoplasms. *Nat. Rev. Cancer* 17, 605–619. doi: 10.1038/nrc.2017.69

Marusyk, A., Tabassum, D. P., Altrock, P. M., Almendro, V., Michor, F., and Polyak, K. (2014). Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity. *Nature* 514, 54–58. doi: 10.1038/nature13556

Merlo, L. M. F., Pepper, J. W., Reid, B. J., and Maley, C. C. (2006). Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer* 6, 924–935. doi: 10.1038/nrc2013

Miller, B. E., Miller, F. R., Wilburn, D., and Heppner, G. H. (1988). Dominance of a tumor subpopulation line in mixed heterogeneous mouse mammary tumors. *Cancer Res.* 48, 5747–5753.

Naffar-Abu Amara, S., Kuiken, H. J., Selfors, L. M., Butler, T., Leung, M. L., Leung, C. T., et al. (2020). Transient commensal clonal interactions can drive tumor metastasis. *Nat. Comm.* 11:1.

Newman, J. C., and Verdin, E. (2017). β-Hydroxybutyrate: A Signaling Metabolite. *Ann. Rev. Nutri.* 37, 51–76. doi: 10.1146/annurev-nutr-071816-064916

Noble, R., Burley, J. T., Le Sueur, C., and Hochberg, M. E. (2020). When, why and how tumour clonal diversity predicts survival. *Evol. Appl.* 13, 1558–1568. doi: 10.1111/eva.13057

Nowell, P. (1976). The clonal evolution of tumor cell populations. *Science* 194, 23–28. doi: 10.1126/science.959840

Ohsawa, S., Sato, Y., Enomoto, M., Nakamura, M., Betsumiya, A., and Igaki, T. (2012). Mitochondrial defect drives non-autonomous tumour progression through Hippo signalling in Drosophila. *Nature* 490, 547–551. doi: 10.1038/nature11452

Poff, A. M., Ari, C., Arnold, P., Seyfried, T. N., and D'Agostino, D. P. (2014). Ketone supplementation decreases tumor cell viability and prolongs survival of mice with metastatic cancer. *Internat. J. Cancer* 135, 1711–1720. doi: 10.1002/ijc.28809

Poff, A. M., Ari, C., Seyfried, T. N., and D'Agostino, D. P. (2013). The ketogenic diet and hyperbaric oxygen therapy prolong survival in mice with systemic metastatic cancer. *PLoS One* 8:e65522. doi: 10.1371/journal.pone.0065522

Quail, D. F., and Joyce, J. A. (2013). Microenvironmental regulation of tumor progression and metastasis. *Nat. Med.* 19, 1423–1437. doi: 10.1038/nm.3394

Robinson, S. P., and Jordan, V. C. (1989). The paracrine stimulation of MCF-7 cells by MDA-MB-231 cells: possible role in antiestrogen failure. *Eur. J. Cancer Clin. Oncol.* 25, 493–497. doi: 10.1016/0277-5379(89)90262-9

Shakery, A., Pourvali, K., Ghorbani, A., Sadat, F. S., and Zand, H. (2018). Beta-Hydroxybutyrate Promotes Proliferation, Migration and Stemness in a Subpopulation of 5FU Treated SW480 Cells: Evidence for Metabolic Plasticity in Colon Cancer. *Asian Pacific J. Cancer Prevent.* 19, 3287–3294. doi: 10.31557/apjcp.2018.19.11.3287

Shimazu, T., Hirschey, M. D., Newman, J., He, W., Shirakawa, K., Le Moan, N., et al. (2013). Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science* 339, 211–214. doi: 10.1126/science.1227166

Soetaert, K., Petzoldt, T., and Setzer, R. W. (2010). Solving Differential Equations in R: Package deSolve. *J. Statist. Software* 33, 1–25. doi: 10.1137/1.9780898719635.ch1

Tabassum, D. P., and Polyak, K. (2015). Tumorigenesis: it takes a village. *Nat. Rev. Cancer* 15, 473–483. doi: 10.1038/nrc3971

Viossat, Y., and Noble, R. (2021). A theoretical analysis of tumour containment. *Nat. Ecol. Evol.* 5, 826–835. doi: 10.1038/s41559-021-01428-w

Vishwakarma, M., and Piddini, E. (2020). Outcompeting cancer. *Nat. Rev. Cancer* 20, 187–198. doi: 10.1038/s41568-019-0231-8

Waclaw, B., Bozic, I., Pittman, M. E., Hruban, R. H., Vogelstein, B., and Nowak, M. A. (2015). A spatial model predicts that dispersal and cell turnover limit intratumour heterogeneity. *Nature* 525, 261–264. doi: 10.1038/nature14971

Wang, Q., Zhou, Y., Rychahou, P., Fan, T. W.-M., Lane, A. N., Weiss, H. L., et al. (2017). Ketogenesis contributes to intestinal cell differentiation. *Cell Death Diff.* 24, 458–468. doi: 10.1038/cdd.2016.142

Yang, J., and Moses, M. A. (2009). Lipocalin 2: A multifaceted modulator of human cancer. *Cell Cycle* 8, 2347–2352. doi: 10.4161/cc.8.15.9224

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frontiers in Ecology and Evolution

HYPOTHESIS AND THEORY
published: 30 July 2021
doi: 10.3389/fevo.2021.682504

# Ecology of Fear: Spines, Armor and Noxious Chemicals Deter Predators in Cancer and in Nature

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In nature, many multicellular and unicellular organisms use constitutive defenses such as armor, spines, and noxious chemicals to keep predators at bay. These defenses render the prey difficult and/or dangerous to subdue and handle, which confers a strong deterrent for predators. The distinct benefit of this mode of defense is that prey can defend in place and continue activities such as foraging even under imminent threat of predation. The same qualitative types of armor-like, spine-like, and noxious defenses have evolved independently and repeatedly in nature, and we present evidence that cancer is no exception. Cancer cells exist in environments inundated with predator-like immune cells, so the ability of cancer cells to defend in place while foraging and proliferating would clearly be advantageous. We argue that these defenses repeatedly evolve in cancers and may be among the most advanced and important adaptations of cancers. By drawing parallels between several taxa exhibiting armor-like, spine-like, and noxious defenses, we present an overview of different ways these defenses can appear and emphasize how phenotypes that appear vastly different can nevertheless have the same essential functions. This cross-taxa comparison reveals how cancer phenotypes can be interpreted as anti-predator defenses, which can facilitate therapy approaches which aim to give the predators (the immune system) the upper hand. This cross-taxa comparison is also informative for evolutionary ecology. Cancer provides an opportunity to observe how prey evolve in the context of a unique predatory threat (the immune system) and varied environments.

**Keywords: predator deterrence, immune evasion, cancer, predator-prey, convergent evolution, armor, spines, noxiousness**

OPEN ACCESS

***Edited by:***
*Elise Huchard, UMR 5554 Institut des Sciences de l'Evolution de Montpellier (ISEM), France*

***Reviewed by:***
*Thomas John Hossie, Trent University, Canada*
*Guy Gilles Beauchamp, Independent Researcher, Montréal, QC, Canada*

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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *18 March 2021*
***Accepted:*** *08 July 2021*
***Published:*** *30 July 2021*

***Citation:***
*Peplinski J, Malone MA, Fowler KJ, Potratz EJ, Pergams AG, Charmoy KL, Rasheed K, Avdieiev SS, Whelan CJ and Brown JS (2021) Ecology of Fear: Spines, Armor and Noxious Chemicals Deter Predators in Cancer and in Nature. Front. Ecol. Evol. 9:682504. doi: 10.3389/fevo.2021.682504*

## INTRODUCTION

Most organisms experience the risk of mortality from predators. In response to this risk, natural selection has imbued prey with a strikingly broad range of anti-predator behaviors, physiologies, and morphologies. One ubiquitous anti-predator defense is fleeing, whether by the legs of a gazelle, the wings of a grasshopper, the flick of a lobster's tail, the hydro-jet propulsion of an octopus, or the flagellum of a single-celled ciliate. Escape into burrows or to refugia inaccessible to predators

also provides safety for many would-be prey, including marine species that escape into rocky interstices to escape predatory fish or into sand to escape shorebirds. Camouflage is another defense pervasive across taxa, which allows prey as diverse as stick-insects, octopuses, and nightjars to blend imperceptibly into the background. In almost all cases, the aforementioned adaptations entail a tradeoff between safety and foraging (McNamara and Houston, 1987; Lima and Dill, 1990; Brown and Kotler, 2004). That is, while fleeing or remaining hidden, an organism must cease feeding; and while actively feeding, it becomes vulnerable.

An intriguing subset of anti-predator adaptations minimizes this tradeoff between foraging and safety, allowing prey to carry on with fitness-enhancing activities even after they are detected by predators. Such defenses include armor, spines, and noxious chemicals. Consider a pangolin digging and foraging at a termite mound. When approached by a lion, it does not have to flee the scene or take refuge. Instead, it can continue foraging, waiting until virtually the last moment of the lion's approach before curling into a ball and defending itself. This provides the pangolin with valuable foraging time. It can remain next to the termite mound and resume foraging as soon as the immediate threat subsides. Critically, many predators will not even attempt to attack the pangolin because of the excessive amount of handling time and effort that would be required to circumvent the armor. This deterrent attribute of armor, spines, and noxiousness may be equally or more important to the prey than the capability of reducing predator lethality in the event of attack.

Here, we focus on the deterrent functions of armor, spines, and noxiousness, which differ somewhat from some other classifications of prey defenses which focus on when in the "predation cycle" a defense is effective (e.g., Jeschke, 2006). We agree that armor affects the prey search step because it increases predator handling time, as well as the final meal step because it decreases predator lethality. Further, we expect that armor will act as a predation deterrent, which should reduce the likelihood of attack. This also applies to spiny defenses. While noxiousness, specifically in the form of toxins, should affect the search step because it increases predator digestion time (Jeschke, 2006), we emphasize that toxins and other forms of noxiousness will serve as deterrents to attack. Warning signals have been identified as affecting the likelihood of attack (Jeschke, 2006), but warning signals are only as useful as the dangerous defenses with which they are associated. As long as predators are foraging optimally, they should be somewhat or completely deterred from attacking armored, spiny, and noxious prey.

Just about all major taxa of living things, from bacteria to single-celled eukaryotes to invertebrates to vertebrates, have members exhibiting armor, spines, noxious chemicals, or combinations of these adaptations (Stankowich and Campbell, 2016; Pančić and Kiørboe, 2018; Klompmaker et al., 2019; Sugiura, 2020). In some cases, possession of one of these adaptations might mean that the other two are unnecessary or can be less pronounced. Slugs without shells or spines may be quite noxious and even poisonous, while snails with shells or sea urchins with their spines are generally not (Lindquist, 2002). In other cases, possessing one of these adaptations, such as armor, may actually amplify the advantages of possessing spines or noxious chemicals (Rice, 1985). This might explain why so many species possess two or all three of these adaptations. Across life forms, armor, spines, and noxiousness display wonderful examples of parallel and convergent evolution.

Though variations of these adaptations are seen across taxa, species exhibiting armor, spines, or noxiousness are usually the exception rather than the rule. This is probably because these adaptations tend to be permanent and costly, more-so than strategies such as camouflage, fleeing, and fixed activity schedules (e.g., nocturnality), which are more common. The production and maintenance of armor, spines, and noxious chemicals all incur an extra energetic and nutritional cost. Exaggerated armor also renders an organism heavier, clumsy, and inflexible. Spines encumber an organism's movements by dragging and snagging on obstacles in the environment. Noxious defenses require the maintenance of specialized physiologies, organs, or diets even if the chemicals are not constantly deployed. Since these defenses are partially to entirely constitutive, if predators are never or rarely encountered, possessing these defenses would be excessively taxing and maladaptive. But, if predators are ever-present, even a costly constitutive defense would be more of an asset than a burden. By allowing would-be prey to continue fitness-enhancing activities in the proximity of aware predators, it essentially gives the prey more enemy-safe space.

Now, consider cancer cells inhabiting their tumor ecosystem. Cancer cells also suffer a form of predation: from the host's immune system. In fact, cancer's ability to evade the immune system may be among its most necessary and ubiquitous features. Immune evasion ranks as a hallmark of cancer (Hanahan and Weinberg, 2011; Fouad and Aanei, 2017). How does this come about?

It might seem that upon initiation the cancer cell would already be immune-evasive, possessing near-identical properties to its progenitor normal cells. But, in transitioning from being part of the whole organism to becoming its own unit of selection, the cancer cell must modify or dispense with several of the properties of normal cells. In becoming its own organism, it must resist programmed cell death, ignore anti-growth signaling and tissue control, and achieve proliferative immortality. Once the cell has become a cancer, natural selection favors adaptations that modify or upregulate intra-cellular metabolic pathways, cell-cell signaling processes, nutrient transporters and membrane pumps, and self-sufficiency in growth factors (Brown, 2016). Heritable variation available to natural selection occurs in cancers via mutations, fixed epigenetic changes, chromosomal rearrangements, copy number variation, and aneuploidy brought on by actual cell fusion or by incomplete cell division (Nam et al., 2020; Pienta et al., 2020b). To be more successful at acquiring nutrients, occupying space, and outcompeting other cancer cells they present antigens to the immune system (Houghton, 1994). In particular, some adaptations of cancer cells result in neo-antigens, novel proteins, and molecules absent from normal cells (Lee et al., 2018). Any of these cancer cell adaptations may invite attack from the immune system. To survive, cancer cells must evolve effective immune evasion (Vinay et al., 2015).

Studies of anti-predator adaptations in nature and cancer have developed along somewhat separate lines. Much of this

difference results from different interests and goals. However, approaches play a large part. Much research has been dedicated to understanding how cancer cells become resistant to the immune system, with the aim of developing immunotherapies to bolster immune system attacks on cancer (Oiseth and Aziz, 2017). A large portion of this work has focused on genes, proteins, and metabolic and signaling pathways that permit cancer cells to avoid detection, attack, or even any response by immune cells. Such genes, proteins, and pathways provide targets for developing chemo- and immunotherapies (Esfahani et al., 2020; Tan et al., 2020). Less has been studied regarding the categories of "anti-predator" behaviors, physiologies, and morphologies of cancer cells. Genes or molecular pathways in cancer may be identified as immunosuppressive without us having knowledge of why the individual cancer cells are in less danger because of these adaptations. Our limited understanding of cancer cell-immune system predator-prey interactions hinders our capacity to anticipate cancer cell responses to altered tissue environments, cell communities, and treatment regimes.

Here we take an ecological perspective on the evolution and utility of different anti-predator adaptations in nature and in cancer (cancer is also a part of nature, but for purposes of terminology we will use "nature" as a shorthand for all other organisms other than cancer). Our reference to the "ecology of fear" is based on the premise that fear is an adaptation for assigning a cost to activities that incur a risk of injury or death. In response to threatening immune cells, cancer cells may be able to evolve some degree of fleeing, hiding, or camouflaging like prey seen in nature, but probably not to the same degree. Furthermore, we shall argue that cancer cells must be able to maintain foraging and proliferation activities in the presence of potentially lethal immune cells. Cancer cells likely enjoy very little enemy-free space. We argue that cancer cells should and do evolve the equivalence of armor, spines, and noxiousness. In fact, these may be some of the most advanced and important adaptations of cancers, evolving again and again in parallel and as convergent evolution from patient to patient, from tumor to tumor within a patient, and perhaps even multiple times among the cancer cells of a tumor.

In what follows, we begin with a discussion of mammals because these species are likely familiar to readers across disciplines; for them, spines, armor and noxiousness are literal both in terms of form and function. We then proceed to describe anti-predator defenses in fishes, insects, and microorganisms; the goal being to transition to taxa that become gradually more similar to single-celled cancer organisms. In this overview, we categorize defenses as being armor-like, spine-like, or noxious. We use these terms because they are easily recognizable in well-known species (e.g., mammals), and therefore serve as convenient references–shorthand, if you will–when we describe systems and traits that are perhaps less familiar to many readers. We categorize defenses into the categories of armor, spines, and noxiousness primarily based on their functions, starting with mammalian examples as a model. We take this approach to emphasize the convergent *functionality* (more-so than appearance) of defenses across taxa.

Next, we briefly detail how the immune system poses threats to cancer cells in terms of types of immune cells, their activation and proliferation, and how they actually kill cancer cells. While the immune system and its cells do not operate under the same ecological and evolutionary principles as predators in nature, they do represent a mortality threat to cancer and exert a selection pressure on cancer cells that is remarkably like that of predators on their prey. We then seek parallel and similar categories of adaptations in cancer that can best be described as armor, spines, and noxiousness. Finally, we note how drawing such parallels between nature and cancer can enrich studies of anti-predator adaptations in nature and suggest ways for how drugs and the immune system can be better deployed to improve patient care.

## MAMMALS

Armored defenses take two main forms in mammals, keratinous scales and osteoderms. Scales are derivatives of hair and are found only in pangolins (Pholidota) (**Figure 1A**). Osteoderms are dermal bone deposits and are (or were) found in plate form in armadillos, glyptodonts, and pampatheres (Cingulata), and as small ossicles in ground sloths (Pilosa) (Hill, 2006). Of these groups, only pangolins and armadillos are extant. Scales and osteoderms often do not provide impenetrable protection against predators' teeth and claws, but they make prey more difficult and time-consuming to kill and ingest. They function as effort deterrents.

In mammals, spiny defenses take the form of modified hairs that are exaggeratedly thick, stiff, and sharp. Taxa with spines (or foam-cored quills) include echidnas (Tachyglossidae), tenrecs (Tenrecinae), hedgehogs (Erinaceinae), Afro-Eurasian porcupines (Hystricidae), and American porcupines (Erethizontidae), with spines evolving independently in each lineage. The extremely long quills of Afro-Eurasian porcupines (**Figure 1F**) and the barbed quills of American porcupines can seriously injure and even kill attacking predators (Afro-Eurasian: Mori et al., 2014; Kerbis Peterhans et al., 2019; Lazzeri et al., 2020; American: Katzner et al., 2015; Elbroch et al., 2016; Forti et al., 2018). These structures will therefore deter predators for risk of physical harm and incapacitation. The relatively short spines of other taxa may not incapacitate predators, and instead, may function like armor by making the prey difficult to handle. Supporting this hypothesis, tenrecs, hedgehogs, and echidnas, which have relatively short spines, will roll up into a ball when threatened, a behavior similar to (armored) pangolins and three-banded armadillos (*Tolypeutes tricinctus*). Spines serve jointly as effort and injury deterrents.

Noxious chemical defenses in mammals include venom, anointed toxins, and foul odors. Slow lorises (*Nycticebus* sp.) produce venom by mixing their saliva with an oily exudate from their brachial glands. They retain the substance in their mouths for a venomous bite or spread it over their fur (Alterman, 1995; Nekaris et al., 2013). Other species "self-anoint" their bodies with toxins or odiferous compounds produced by other organisms. African crested rats (*Lophiomys imhausi*) chew the bark of African poison arrow trees (*Acokanthera schimperi*), then apply

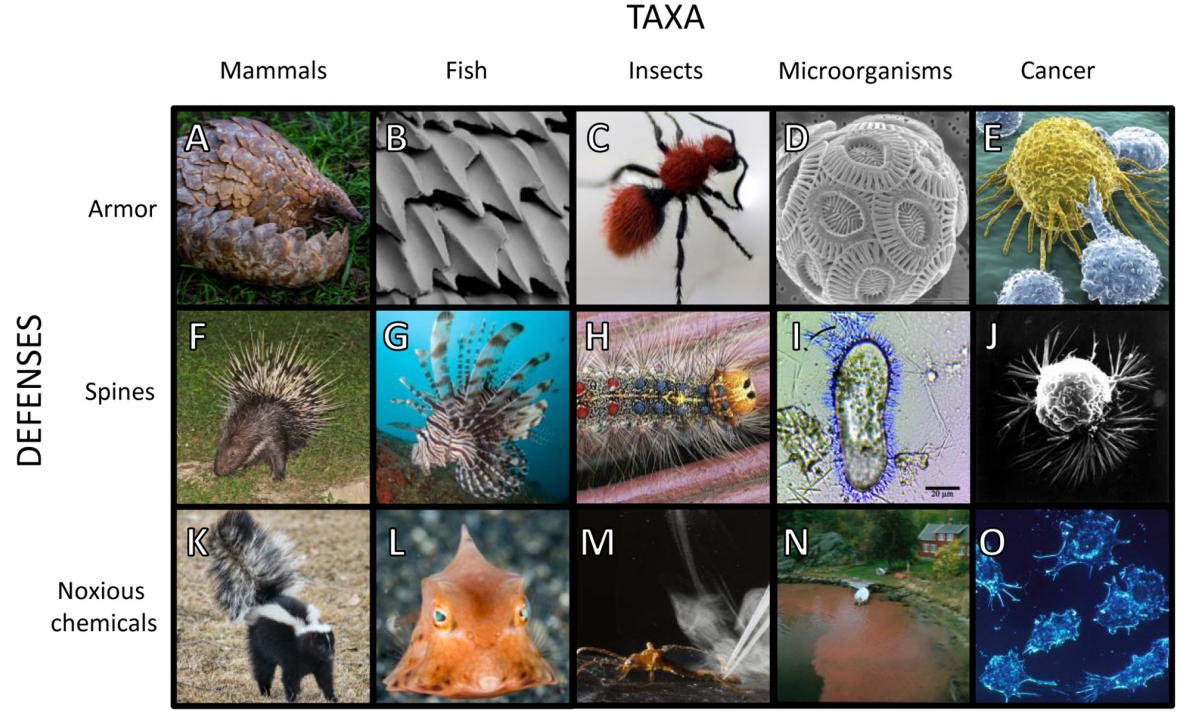


**FIGURE 1 |** Images of species with armor, spines, and noxious chemical defenses. Examples of armor: **(A)** Temminck's ground pangolin (*Smutsia temminckii*) (Wursten, 2017); **(B)** scanning electron micrograph of placoid scales on a white shark (*Carcharodon carcharias*) (Lindsay, 2019); **(C)** velvet ant (Mutillidae) (Pemberton, 2009); **(D)** scanning electron micrograph of coccolithophore (*Emiliania huxleyi*) (Taylor, 2011); **(E)** cancer cell being attacked by T-cells (Gartner, 2021). Examples of spines: **(F)** Malayan porcupine (*Hystrix brachyura*) (Villemagne, 2008); **(G)** lionfish (*Pterois* sp.) (Mistry, 2019); **(H)** *Lymantria dispar* (Materialscientist, 2009); **(I)** *Paramecium tetraurelia* with trichocysts (DavidpBowman, 2014); **(J)** scanning electron micrograph of breast cancer cell (Wetzel and Schaefer, 1980). Examples of noxious chemical defenses: **(K)** striped skunk (*Mephitis mephitis*) (Keck, 2011); **(L)** thornback cowfish (*Lactoria fornasini*) (Zerpe, 2018); **(M)** bombardier beetle (*Brachinus elongatulus*) (Hedgcock, 2013); **(N)** red tide *Dinophysis* bloom (Dahl, 2019); **(O)** cancer cells impeding immune cells by creating an acidic environment (Fox, 2001). Images were cropped but not otherwise altered.

the toxic material to specialized hairs on their flanks (Kingdon et al., 2012; Weinstein, 2020; Weinstein et al., 2020). Interestingly, African crested rats also have armor-like traits. Their reinforced skulls and remarkably tough skin are resilient to "all but the sharpest of teeth, claws or beaks" (Kingdon et al., 2012).

Odiferous anal-gland secretions and urine are widespread across mammal species and are often used for scent marking and communication (Mengak, 2005; Stankowich et al., 2011; McLean, 2014; Jansen et al., 2020). Skunks (**Figure 1K**) and stink badgers (Musteloidea: Mephitidae) and striped polecats (Musteloidea: Mustelidae) have co-opted anal secretions for defense (Stankowich et al., 2011). When sprayed on potential predators, a skunk's thiol-containing musk causes a burning sensation in the eyes (Cuyler, 1924; Wood et al., 2002). Skunks, stink badgers, striped polecats, slow lorises, and African crested rats all have black and white aposematic coloring which warns predators of their noxiousness (Stankowich et al., 2011; Nekaris et al., 2019). Noxiousness serves as an injury deterrent.

Several characteristics unite mammals with armored, spiny, and noxious defenses. Relative to the mammalian norm, mammals with spines, armor, and noxious chemicals exhibit locomotion associated with stability rather than speed (Lovegrove, 2001). Furthermore, they often have low metabolic rates (McNab, 1984, 2008; Haim et al., 1990; Stephenson and Racey, 1994; Lovegrove, 2000). While attributing causation can be tricky, armor, spines, and noxiousness may both be associated with and permit slower speeds and lower metabolic rates. Most intriguing and compelling is that these defenses (unlike fleeing, camouflage, and escape to refugia) allow the mammal to maintain feeding activities in the presence of predators that have detected them, even at close proximity. Approach distance (or flight initiation distance) describes how close a predator can get to a prey before it flees. We posit that armor, spines, and noxiousness significantly reduce approach distances while discouraging predators from approaching at all. This theme will be repeated in the following sections.

## FISHES

Armor and spines occur early in fish evolution. Extinct jawless fishes in the clade Osteostraci exhibited a conspicuous armored endoskeleton headshield, with plates and spines (Klompmaker et al., 2019). With the evolution of jaws, gnathostome fish emerged as predators of other fish. Their prey often evolved armor in defense. Extinct placoderms possessed articulated armored plates that covered their head and body

(Klompmaker et al., 2019). Cartilaginous fishes (Chondrichthyes; sharks, skates, and rays) have armored placoid scales (**Figure 1B**; Raschi and Tabit, 1992). Extinct lobe-finned fishes (Sarcopterygii) were covered in tough bone and keratin and extant coelacanths (Coelacanthidae) have specialized scales that are resistant to damage from predators (Quan et al., 2018). Ganoid scales in Polypteriformes (bichirs) and Lepisosteiformes (gars), and bony plates in Acipenseriformes (sturgeons) also provide armored deterrence (Song et al., 2011; Yang et al., 2013; Wishingrad et al., 2015). Armature is persistent throughout spiny ray-fined fishes (Percomorpha) such as boxfishes (Ostraciidae), which have dermal scutes that provide protection from both penetrating and crushing forces (Yang et al., 2015). Even the seemingly inconspicuous scales of small mouth bass (*Morone saxatilis*) provide protection from predators by resisting punctures (Zhu et al., 2012). The effort deterrence of armor gives way to injury deterrence when these features are also spiny or associated with spines.

Being spiny allow fish to evade predation by threatening harm, preventing capture, increasing handling time, or otherwise reducing predator efficiency (Forbes, 1989; Nilsson et al., 1995; Nilsson and Brönmark, 2000). Head and fin spines and deep bodies are common defensive traits. Many piscivores are gape-limited predators, therefore a deep body effectively frees the prey from these predators. In fact, spines may have evolved with deepening or widening body shape for this very reason (Price et al., 2015). Spines may be erected as needed to increase body depth in groups like triggerfish and filefish (Balistidae and Monacanthidae). Other Tetraodontiformes (including porcupinefish, Diodontidae, and pufferfish, Tetraodontidae) possess a behavioral startle response to predation threats that includes increasing body size and erecting body spines (Greenwood et al., 2010; Pleizier et al., 2015). The evolution of fin spine length in butterflyfish (Chaetodontidae) correlates with foraging in risky habitats and situations (Hodge et al., 2018). Spines serve jointly as effort and injury deterrents.

Noxious slimes, venoms and toxins occur frequently throughout the evolutionary history of fishes. Pre-vertebrates and jawless fishes (hagfishes, Myxinidae) deploy a noxious slime that smothers the gills and suffocates would-be predators (Zintzen et al., 2011). Venom is observed in at least 58 fish families and serves both predatory and anti-predatory functions (Smith et al., 2016; Harris and Jenner, 2019). In some species, modified fin and body spines form hypodermic needles capable of injecting venom into predators (Harris and Jenner, 2019). This defense is hypothesized to contribute to lionfishes' (*Pterois miles* and *Pterois volitans*) (**Figure 1G**) wide niche breadth and *P. volitan's* expansive range and success as an invasive species (Harris and Jenner, 2019). Noxious chemical defenses can take the form of ichthyotoxins which are secreted from the skin and aid in escape. To reduce parasitic load or when threatened, pufferfish (Tetraodontidae) release tetrodotoxin through their skin (Saito, 1985; Munday et al., 2003). Boxfish and cowfish (Ostraciidae) (**Figure 1L**) release ostracitoxin as a poisonous secretion (Thomson, 1964). Notably, both these families are comprised of relatively slow swimming omnivores found on coral reefs.

Conspicuous defensive traits such as armor, spines, or noxious chemicals play a large role in fish behavior and foraging. Fish without defensive armor mitigate predation risk with behavioral responses such as fleeing (McLean and Godin, 1989). Consequently, unarmored fish may reduce the amount of time foraging and flee sooner than those that are defended. Armored stickleback (*Culaea inconstans*) preferred to associate with non-defended fathead minnows (*Pimephales promelas*) in high-risk environments, perhaps because they were the predator's less preferred prey (Mathis and Chivers, 2003). Such tradeoffs between foraging and predation risk have evolutionary consequences. For example, butterflyfish species (Chaetodontidae) with longer spines have riskier foraging strategies such as being solitary or venturing farther from the safety of the reef (Hodge et al., 2018). As with mammals, armor, spines, and noxious chemicals in fish may permit tenacious foraging under high risk, permit closer approach distances, and discourage predators from attacking at all.

## INSECTS

Insects have evolved a spectacular array of defenses. Sugiura (2020) classified insect defenses into chemical, morphological, physical, and behavioral categories. In a manner similar to the other taxa we discuss, Sugiura's morphological and physical categories include armor and spines, and chemical and behavioral categories include toxic or noxious exudates, venoms, and regurgitated gut fluids.

An insect's exoskeleton acts as armor among other functions (Davies, 1988). As body armor, the exoskeleton is made of stiff sheets or lamellates of chitinous and proteinaceous material connected by a flexible membrane which allows the entire exoskeleton to move (Waldbauer, 2012). In some insect species, the chitinous exoskeleton can be so hard that it is all but impervious to crushing and digestion. A striking example first observed by Alfred Russel Wallace (Wallace, 1867, 1895, as seen in Wang et al., 2018b) are the Pachyrhynchus weevils (Coleoptera: Curculionidae: Entiminae: Pachyrhynchini). The exceptional strength of mature weevil exoskeletons results from a thickly sclerotized cuticle, combined with fibrous ridges in the endocuticle layer of the exoskeleton, apparently unique to these weevils (Wang et al., 2018a). Such defenses act primarily as an effort deterrent.

Many insects have evolved defensive hairs (setae) and spines. The lubber grasshopper (*Romalea guttata*) has sharp spines on its hindlegs which deter predators (Eisner et al., 2005). Many lepidopteran taxa possess hairs and spines. Some of these are "urticating" or stinging structures, while others, e.g., those of the mulberry tiger moth (*Lemyra imparilis*) and the moth, *Lymantria dispar* (**Figure 1H**), provide only a physical deterrence to predators (Whelan et al., 1989; Sugiura and Yamazaki, 2014) or parasites (Kageyama and Sugiura, 2016). On at least some species, the hairs increase in length and/or density in later larval instars, and these developmental changes appear to increase the deterrence effect of the hairs (Whelan et al., 1989; Sugiura and Yamazaki, 2014).

Insects manifest an extraordinary diversity of chemical defenses (Eisner, 1970; Blum, 1981; Eisner et al., 2005). These defenses are found on the surface, in blood, the gut, or systemically (Eisner et al., 2005). Glandular chemical defenses may be injected into the enemy or secreted in other ways (Eisner, 1970). In some species, venoms may be used both for defense and to acquire prey. Finally, chemical defenses may be produced endogenously or acquired exogenously (Eisner et al., 2005). Many herbivorous insects sequester plant secondary compounds intended for defense against herbivores. In these cases, specialized herbivores are often better defended against their own predators than are generalist herbivores (Zvereva and Kozlov, 2016).

Given their soft bodies, larvae of Coleoptera and Lepidoptera are particularly vulnerable to physical attack, and, unsurprisingly, many are well defended by spines (brushy setae) or noxious chemicals. The unicorn caterpillar moth (*Schizura unicornis*) sprays its defensive chemical cocktail from a saclike gland located behind the head (Eisner et al., 2005). The bombardier beetles (Coleoptera) (**Figure 1M**) produce their defensive chemicals, benzoquinones, which combine explosively when ejected. The reactants, hydrogen peroxide and hydroquinones, are forced through a "reaction chamber," where catalases and peroxidases drive the chemical reaction, ejecting "their spray at the temperature of boiling water" (Eisner et al., 2005, p. 159–160). Their spray repels spiders, ants, frogs, and birds.

Insects commonly have two or even all three forms of effort/injury deterrents. Velvet ants (Hymenoptera: Mutillidae) (**Figure 1C**) possess a suite of formidable defenses, including aposematically colored, coarse, dense hair (setae), a chemical alarm signal, stridulatory warning sounds, and potent stings (Hertz, 2007; Schmidt, 2016). In addition, they are protected by a round, slippery, and extremely hard exoskeleton (Schmidt and Blum, 1977; Gall et al., 2018). They can even survive over 20 min in the stomach of a toad prior to rejection by regurgitation (Mergler and Gall, 2021).

Many insect species flee at the approach of a potential predator (e.g., cockroaches, houseflies, and grasshoppers), exhibit amazing camouflage (e.g., peppered moth, stick insects, planthoppers), or overwhelm their predators numerically with occasional emergences (e.g., periodical cicada, mayflies) or outbreaks (swarming locusts). We hypothesize that these species are less likely to exhibit armor, spines, and noxious chemicals. Such species should be more likely to cease feeding activities at the approach of predators. Insects that use effort/injury deterrents pay a price for their defenses (Flenner et al., 2009) but can maintain activities in the presence of predators (Witz and Mushinsky, 1989; Ge et al., 2019). In fact, most insects that seem "easy" to catch are likely defended with some combination of armor, spines, and noxiousness (or sheer numbers).

## MICROORGANISMS

We see cancer as a speciation event in which a protist evolves from the cells of its host (Gatenby and Brown, 2017; Gatenby et al., 2020; Pienta et al., 2020a). From this perspective, microorganisms provide the closest examples for cancer of armored, spiny, and noxious defenses, both in form and function. The term "microorganism" includes archaea, bacteria, protozoa, fungi, and algae. For this diverse polyphyletic grouping, our goal is to highlight how and when armored, spiny, and noxious defenses are seen in unicellular organisms.

Armor-like defenses in unicellular species include reinforced cell walls (armored plates and sheaths) and robust extracellular matrices. In phytoplankton (single-celled algae), these provide effort deterrence from zooplankton grazing (Hamm et al., 2003). Most species of dinoflagellates have cell walls surrounded by armor-like cellulosic sheaths known as thecae. Diatoms have frustules, silicified cell walls (Hamm et al., 2003), which allow them to survive passage through a predator's gut (Fowler and Fisher, 1983). Diatoms thicken their frustule walls in response to copepod grazing (Pondaven et al., 2007; Grønning and Kiørboe, 2020). Coccolithophore phytoplankton are named for their calcium carbonate plates, or coccoliths, which surround their cell walls (**Figure 1D**). Heterotrophic protist predators exhibit significantly reduced population growth rates when fed calcified rather than non-calcified (less armored) strains of *Emiliania huxleyi* coccolithophores (Harvey et al., 2015; Pančić and Kiørboe, 2018). *E. huxleyi* have a haplo-diplontic life cycle and only the diploid cells, which are non-motile, are calcified (Kolb and Strom, 2013). This is consistent with our hypothesis of a relationship between armored defenses and less mobile (or sessile) lifestyles.

Cell and colony size and shape also influence host-grazer interactions. Prey morphologies that are large or unwieldy increase handling time for predators and thereby function as armor despite the lack of a thick outer covering. The presence of some species of *Daphnia* (zooplankton grazers) will induce populations of *Scenedesmus* phytoplankton to transition into linked-up colonies with spiny morphologies (van Donk and Hessen, 1993; Pančić and Kiørboe, 2018). Lürling et al. (1997) confirmed that *Daphnia* species grazed *Scenedesmus* at lower rates when *Scenedesmus* formed large, linked colonies. Similarly, protozoan grazers promote planktonic bacteria with elongated filamentous morphologies (Jürgens et al., 1994; Pernthaler et al., 1996; Hahn et al., 1999), which can manifest as individual cells with strongly elongated shapes or thin chains of multiple cells (Hahn et al., 1999). Various bacterial species are permanently or facultatively filamentous, but the increased prevalence of filamentous bacteria in the presence of protozoan grazers reflects differential survival, not an induction of filamentous morphology (Hahn et al., 1999).

Another type of armor-like defense in microorganisms is observed in biofilms, which adhere together by extracellular matrices of polymeric substances (polysaccharides, nucleic acids, and proteins). The exopolymeric matrix constitutes a "biofilm shield" that is difficult for predators to penetrate, and the unwieldy masses of cells resist phagocytosis by unicellular predators (Matz and Kjelleberg, 2005). Phagocyte predators are less hindered when this shield is broken. For example, neutrophils have an elevated response (oxidative burst) to *Pseudomonas aeruginosa* surface biofilms when they have been mechanically disturbed (Kharazmi, 1991; Jensen et al., 1992). Biofilms are

effective at defending against suspension-feeding protozoans in aquatic/marine habitats (Matz et al., 2002; Seiler et al., 2017), and immune cells such as neutrophils and macrophages in host systems (Jesaitis et al., 2003; Chandra et al., 2007; Thurlow et al., 2011; Roilides et al., 2015).

Spines and distinctive adaptations that function like spines provide microorganisms with safety from predation through both effort and injury deterrence. Some ciliates and dinoflagellates produce filamentous trichocysts, silicified needle-like extrusive organelles (**Figure 1I**). When triggered, these protists discharge a barrage of trichocysts outside their cells, creating a spikey obstruction between the protist and its predator (Knoll et al., 1991). To avoid injury, a persistent predator must detour around the trichocyst mass, resulting in increased subduing time. The dinoflagellate *Prorocentrum micans* possesses both trichocysts and armor-like theca (Rhiel et al., 2018). The diatom *Chaetoceros peruvianus* produces spine-like extensions of its already armor-like siliceous frustule. These extensions (setae) are believed to deter predators by increasing handling effort (Pickett-Heaps et al., 1994; Smodlaka Tanković et al., 2018).

Toxins and other noxious chemical defenses are employed by diverse species of archaea, bacteria, phytoplankton, and yeasts. Perhaps the most infamous are the neurotoxins of dinoflagellates, diatoms, and cyanobacteria, which can lead to harmful algal blooms or red tides. These neurotoxins include chemicals of the saxitoxin family, commonly known as paralytic shellfish toxins, as well as spiroimines, goniodomin A, and lytic compounds (Xu et al., 2017). Most of the noxious chemicals of phytoplankton are retained in their cells, delivered to predators only upon ingestion. Some *Alexandrium* species also produce extracellular allelopathic compounds (Ma et al., 2009). Harmful algal blooms can originate from several genera of dinoflagellates, including *Dinophysis*, shown in **Figure 1N**.

Noxious chemical defenses are commonly deployed by yeast (and other fungi) and bacteria, especially in biofilms. Because fungi, including yeasts, are generally immobile, high-nutrient patches are desirable and competition for them can be fierce. Fungi have thus evolved to produce an array of noxious chemicals which they employ against bacterial and other fungal competitors (Künzler, 2018). They also provide injury deterrence against predators. In yeasts, these substances, known as killer toxins, are secreted from the cell. Some species of marine bacteria produce the alkaloid compound violacein, which induces a cell death program in protozoan predators (Matz et al., 2008). These bacteria do not excrete the violacein, but retain it in their cells, making them toxic upon consumption. Matz et al. (2008) found that violacein production per bacterium was 3–59 times higher for cells in biofilms than planktonic cells.

As they do for multicellular species, armored, spiny and noxious defenses allow microorganisms to defend in place, a behavior that is especially useful when foraging from resource-rich patches, or when a microorganism is sessile. The advantage of using effort or injury deterrents to remain active, in place, is especially evident when considering biofilms. Bacteria can reach much higher densities in biofilms than in the water column (Matz et al., 2008). Some researchers have hypothesized that these high cell densities can be reached because biofilms provide refuge from predators (Matz and Kjelleberg, 2005), and others have noted that surfaces tend to concentrate nutrients (Baty et al., 2000; Hall-Stoodley et al., 2004). We expect that these two conditions are not coincidental, but closely related. Planktonic bacteria can escape predators by fleeing, but this is an undesirable strategy when swimming away from a nutrient-rich patch.

In response to the immune system, do cancer cells evolve some subset of armor, spines and noxious chemicals as seen among microorganisms?

## THE IMMUNE SYSTEM AS A PREDATOR

The National Cancer Institute describes the immune system as "a complex network of cells, tissues, organs, and the substances they make that helps the body fight infections and other diseases" (NCI, 2021). The human immune system is a marvel with layers of complexity for the simple purpose of killing pathogens and pathogen-infected normal cells, and for removing debris and malfunctioning cells. It involves a large number of cell types, diverse signaling molecules, and a variety of spatial scales near and far from the actual location of infection or attack. It provides both surveillance, memory, adaptability, and attack. Specialized cells of the immune system, particularly killer T-cells, do threaten and kill cancer cells. To the cancer cells, they exert selection pressures just like predators on their prey in nature.

In this section we describe some of the features of the immune system and highlight the striking differences between cytotoxic immune cells and most predators in natural systems. Compared to traditional predators, they should not and do not respond in the same way to effort and injury deterrents from their pathogen prey. However, the immune system does drive the evolution of immune evasion and suppression by the cancer cells within the patient, and the suite of adaptations deployed by cancer cells can, in many cases, resemble the effort and injury deterrents described for the other taxa in function if not always in form.

The cancer cells face threats from the innate and the adaptive immune system, though the latter likely exerts stronger selection on the cancer cells' anti-immune adaptations. But, the innate immune response of natural killer (NK) cells, macrophages, and dendritic cells may be crucial for priming the adaptive response that includes antigen presenting cells (APC) and cytotoxic T-cells. NK cells can kill from a distance by releasing proteases and other substances in close proximity to the cancer cell. These substances puncture holes in the cancer cell's membrane and permit additional lethal proteases to enter and kill the cancer cell. T-cells operate similarly, but they must be in contact with the cancer cell. Macrophages kill cancer cells by engulfing them through phagocytosis. In the tumor, macrophages and NK-cells, through their killing of cancer cells, and APC and dendritic cells, through their transport of bound antigens to lymph nodes, can recruit, prime, and activate cytotoxic T-cells. The T-cells flow through the blood to the tumor from the lymph nodes, where they can continue to proliferate as well as attach to cancer cells (Figure 1 in Demaria et al., 2019 provides an excellent illustration).

There are three noteworthy features of the interaction of the immune system with cancer cells. First, while it is not a predator-prey system in the usual sense, the action of cytotoxic T-cells can be conceptualized and modeled as a predator (de Pillis et al., 2005; Kareva et al., 2010; Robertson-Tessi et al., 2012; Kaur and Ahmad, 2014). As a "predator," T-cells are subsidized in the sense that new ones can be recruited from the lymph nodes, independent of killing rate and success (Blank et al., 2016). This could be compared to house cats (T-cells) preying on song birds (cancer cells) where the fate of the cat population is uncoupled from the fate of the song bird population because the cats are supported by pet food (Lepczyk et al., 2004). Second, as modeled and noted by Kareva et al. (in review), cytotoxic T-cells do not work on commission like natural predators. Their proliferation can be stimulated by the overall presence of the antigen and the number of cancer cells. But, a given cytotoxic T-cell's proliferation rate does not increase with the rate at which it kills cancer cells. In fact, in a phenomenon known as T-cell exhaustion (Zarour, 2016; Wang et al., 2018; Philip and Schietinger, 2019), a T-cell may become injured and incapacitated in the process of attaching to and killing a cancer cell. Hence, the direct effect of T-cells on cancer cells, and vice-versa, bears a greater resemblance to extreme interference competition than predation (Kareva et al., in review). Third, other immune cells such as regulatory T-cells (T-regs) act as a break on the proliferation of cytotoxic T-cells. Their proliferation is stimulated by cytotoxic T-cells even as T-regs suppress the proliferation of cytotoxic T-cells. In its simplest guise, T-regs, cytotoxic T-cells and cancer cells form a kind of tri-trophic level system: T-regs "prey" upon and benefit from T-cells, and T-cells prey upon and benefit from cancer cells (Dullens et al., 1986; Eftimie et al., 2016; Walker and Enderling, 2016).

In the previous sections we discussed examples of species that defend themselves from predators for whom successful foraging leads to increased individual fitness. Like other organisms, these predators make foraging decisions that balance nutritional and energetic rewards against the risk of bodily harm and missed opportunity costs. We shall refer to these types of predators as traditional predators. Immune cells fall into a different category of predator. While they are predators insomuch as they exert selection on prey (pathogens, cancer cells) as traditional predators would, cytotoxic immune cells are not traditional predators. They do not individually benefit from successful foraging (killing cancer cells). This is because the unit of natural selection is not the individual immune cell but the entire host organism. Immune cells' behaviors serve to benefit the entire organism, even at the expense of the individual immune cell. In this way, they may be likened to the soldier caste of a eusocial species, such as soldier ants, which will walk into the face of danger for the benefit of the colony.

For systems with traditional predators, armor-like defenses are effective primarily as an effort deterrent. All else equal, traditional predators will opt for the easier (unarmored) prey. This strategy maximizes time and energy efficiency. Soldier-caste-type predators, meanwhile, may not be deterred from pursuing difficult prey. Their preference should reflect the needs of the whole organisms or colony. For example, neutrophils and macrophages attempt to phagocytize biofilms even when they are too large to engulf, which frustrates phagocytosis (Leid, 2009; Thurlow et al., 2011). Prey defenses that incapacitate predators function as injury deterrents. When alternatives are available, traditional predators will opt for less dangerous prey. When the fitness reward from a prey does not counterbalance the risk of lethal or incapacitating injury to the predator, then the predator should forgo that prey entirely. Soldier-caste-type predators, meanwhile, will not be deterred from pursuing dangerous prey, even if there is a high probability that the soldier will be killed. Attempts by neutrophils and macrophages to engulf bacterial biofilms will trigger the bacteria to release cytotoxic chemicals that are effective in killing the cells (Thurlow et al., 2011; Hirschfeld, 2014; Scherr et al., 2015). These phagocytes lose twice, first by the prey's armored defense and second by the prey's noxious defense, yet they still pursue the prey until their deaths. **Figure 2** summarizes how defenses that either increase subduing-handling time or threaten incapacitation will affect traditional and soldier-caste-type predators. Though soldier-caste-type predators will not necessarily be deterred for their own self-preservation, they can be deterred for the sake of the whole organism's or eusocial colony's wellbeing.

Self-attack, where the attacker misidentifies benevolent cells or individuals of the (super)organism as threats, is a particularly relevant concern for soldier-caste-type predators. In eusocial animal colonies, discernment of colony members decreases the chance of both self-attack (Michener, 1974; Crosland, 1990; Fishwild and Gamboa, 1992), which is analogous to autoimmunity, and parasitism, which is analogous to infection or cancer. Naked mole rats, which are eusocial, use odors to recognize colony members, with individuals' odors mixing to create a unique and dynamic colony scent (O'Riain and Jarvis, 1997). Almost universal among eusocial insects (Breed and Bennett, 1987; Smith and Breed, 1995), nest mate recognition is accomplished in a similar way, by picking up the chemical profile unique to the colony with their antenna. This strategy is useful for detecting colony threats such as parasites, unless the parasites can convincingly mimic colony members or the nest itself. Eusocial stingless bees (*Melipona subnitida*) will swiftly attack and kill full adult (post-eclosion) parasitic mantisflies (*Plega hagenella*) that enter their colony but will not kill younger adults (pharates) still in pupa (Maia-Silva et al., 2013). Perhaps because the pharates convincingly mimic the scent profile of the nest, the bee workers simply gently remove the pharates with the nest's waste, at which point the mantisflies continue their life cycle (Maia-Silva et al., 2013). Maia-Silva et al. (2013) conclude that delayed adult eclosion in these mantisflies is an important adaptation to avoid attack by host bees.

Immune cells depend on antigen recognition to discern self and non-self. To evade immune cell predation, cancer cells should disguise themselves as host cells, similar to the strategy of the parasitic mantisfly pharates. However, this presents a tradeoff to the cancer cells. Novel adaptations that would make them more successful at acquiring nutrients, occupying space, and outcompeting other cancer cells–e.g., modification or upregulation of intra-cellular metabolic pathways, cell-cell signaling processes, nutrient transporters, membrane pumps, and self-sufficiency in growth factors (Brown, 2016)–will also

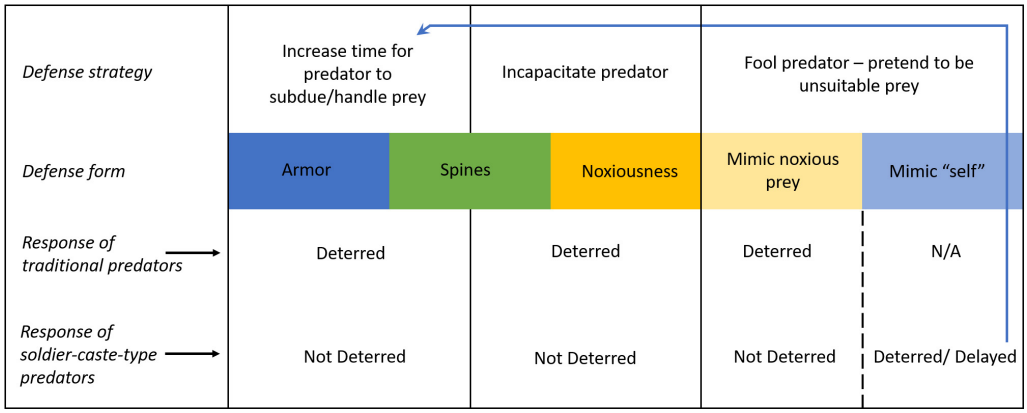


**FIGURE 2 |** Defenses which function to increase predator handling time and/or subduing time (armor and, in some instances, spines) will deter traditional predators but not soldier-caste-type predators. Defenses which incapacitate predators (noxious chemicals and, in some instances, spines) will also deter traditional predators but not soldier-caste-type predators. Prey can also fool predators with false signals, pretending to be unsuitable prey (Batesian mimics). For traditional predators, noxious prey are unsuitable prey. Mimicking noxious prey will deter traditional predators but not soldier-caste-type predators. For soldier-caste-type prey, "self" cells/individuals from the same organism/superorganism are unsuitable prey. Mimicking "self" cells/individuals could deter soldier-caste-type predators, or simply increase the time required for proper identification. By delaying proper identification, these mimics are effectually increasing predator subduing time, making this an armor-like defense. For traditional predators not part of a superorganism, the distinction between self and non-self is obvious so self-mimicry is N/A.

result in conspicuous antigen presentation (Houghton, 1994; Lee et al., 2018). Having supplementary anti-predator defenses such as armor, spines, or noxious defenses could allow cancer cells to incorporate novel adaptations with impunity, even if the accompanying antigens ultimately increase immune cell attack.

The adaptive immune system creates a coevolutionary arms race between cancer cells and the host immune system which bears some similarities to that between traditional prey and predators (Kareva et al., in review). This arms race reoccurs *de novo* within each cancer patient. In traditional predator-prey models, predators directly convert consumed prey into more predators (predator biomass), but this is typically not the case for immune cells (Merlo et al., 2006, Kareva et al., in review). However, the adaptive immune system will produce cytotoxic T-cell variants that successfully target invader cells (Merlo et al., 2006). The direct conversion of prey into predator biomass has evolutionary consequences because successful predators will have more offspring, selecting for superior predatory traits. Within the lifespan of an individual host, cancer cells have the opportunity to evolve immune evasion over many generations, but the host does not. The adaptability of the immune system enables the host to modulate how it attacks the changing cancer cell community.

Cancer cells subjected to NK cells, macrophages, cytotoxic T-cells and other associated regulator cells find themselves being the prey, so their immune evasion responses are akin to standard anti-predator adaptations that emerge from traditional predator-prey systems. Tumors can be classified as hot versus cold depending upon the amount of immune infiltration (Maley et al., 2017; Vareki, 2018), and hot tumors are thought to be more responsive to immunotherapies that challenge or target the cancer's anti-predator adaptations (de Guillebon et al., 2020). While often novel in form, cancer cells' anti-predator adaptations against the immune system function much like armor, spines, and noxiousness in other species.

## ANTI-PREDATOR ADAPTATIONS IN CANCER

As noted by Fridman (2018), the observation that the immune system might suppress cancers dates back to 1891 (Coley, 1891). However, the immune system's therapeutic value did not become fully appreciated until this century. Not until 2011, did Hanahan and Weinberg add immune evasion to their original 2000 "hallmarks of cancer" (Hanahan and Weinberg, 2000, 2011). The field of cancer biology has progressed from noting how cancer cells may have adaptations to avoid the immune system to accepting that all successful cancer cells possess one or more evasion strategies. NK cells, macrophages, cytotoxic T-cells, and more are an ever-present feature of tumors, even in cold tumors or regions of a tumor where immune infiltration is weak. Cancer cells must and do maintain feeding, normal activity, and proliferation while surrounded by threats from immune cells. Cancer cells cannot truly flee, hide, or remain camouflaged (entirely unnoticed) from immune attack. For all these reasons, they need armor, spines, and noxiousness for defense. Though these adaptations can take on forms quite different from those in other taxa, they still function to deter "predators," in this case predatory immune cells.

As the number of cancer cells grows, natural selection promotes increasingly effective anti-predator adaptations, thus tipping the scales in favor of the cancer cells and against the immune system (Solinas et al., 2009). This temporal progression toward cancer cells winning the arms race is termed cancer immunoediting (Shankaran et al., 2001). It has three recognized phases (Dunn et al., 2004; Pandya et al., 2016). In the first (elimination phase), the cancer cells are so few and so vulnerable that the immune system can eliminate them completely (Burnet, 1957; Corthay, 2014). In the second (equilibrium phase), the number of cancer cells and the sophistication of their immune

evasion adaptations result in an equilibrium where the tumor is neither growing nor shrinking or being eliminated by the immune system (Koebel et al., 2007; Teng et al., 2008). In the third (escape phase), the number of cancer cells and their adaptations allow them to thrive and expand their range (tumor growth and metastases) even in the face of a fully functioning immune system (Khong and Restifo, 2002; Grivennikov et al., 2010).

There are three general ways by which cancer cells evade the immune system (Wildes et al., 2020). First, cancer cells alter surface membrane molecules that fool cytotoxic T-cells into perceiving them as unsuitable prey. Second, cancer cells modify their extracellular environment in a manner that repels immune cells or renders them less effective. Third, cancer cells release or present molecules that render immune cells inoperable or that alter the immune cell composition from one that is tumor-suppressive to one that is pro-tumor (Mohme et al., 2017).

Whereas natural predators try not to waste time on unprofitable prey, cytotoxic immune cells go out of their way to avoid killing healthy, normal cells. Armor–in the functional sense of increasing handling time–for cancer cells takes the form of increasing the time required for cytotoxic immune cells to recognize the cancer cell as prey (non-self), even to the point of ceasing to see the cancer cells as anything but a normal cell to be avoided (**Figure 2**). Cancer cells acquire this armor by changing surface proteins, altering the expression of MHC molecules, down-regulating NK cell activating ligands, or simply forgoing the advantages of antigen presenting membrane properties (Beatty and Gladney, 2015; Steven and Seliger, 2018; Anichini et al., 2020). "Armor" by this interpretation is no longer a barrier that frustrates physical processing by the predator, but now a barrier that frustrates diagnosis processing by the predator. This is because only the latter type of barrier will be effective at deterring predation by immune cells. This defense by the cancer cells can also be recognized as a form of mimicry.

A fascinating example of an armor-like immune escape comes from a mouse model of adoptive cell transfer therapy (ACT) against melanoma. In this model, an infusion of T-cells specifically recognizes a melanoma differentiation antigen, gp100, leading the melanoma cells to adapt by decreasing expression of gp100 and switching to a less differentiated neural crest phenotype (Landsberg et al., 2012). This response is mediated by TNFα, released by tumor-infiltrating cells as a part of a normal immune predation program. A downregulation of gp100 is accompanied by the expression of the nerve growth factor receptor (NGFR) and the loss of the expression of several melanosomal antigens. Thus, the cancer cells mimic embryological tissue. Even as the immune cells constantly encounter these cancer cells, they perceive them as unsuitable prey.

Cytotoxic immune cells are not completely devoid of behaviors of self-preservation. They will avoid prey perceived as "self" and they will avoid toxic circumstances. Spines to a cancer cell can be literal protrusions that prevent cytotoxic T-cells from contacting the cell membrane, or, more frequently, defenses that function like "spines." Cancer cells do this via changes to the microenvironment that cause cytotoxic immune cells to avoid approaching the cancer cells. Literal spines or protuberances appear in single cell microscopy of cancer cells (**Figure 1J**). They may be invadopodia facilitating collagen degradation within the extracellular matrix (Weaver, 2006; Augoff et al., 2020), pseudopodia for movement (Guirguis et al., 1987), or extracellular extensions of intermediate filament proteins (usually associated with the cell's cytoskeleton) that may serve for immune evasion (Sharma et al., 2019).

At present, the role of such true spines and filaments is poorly studied. How cancer cells generate microenvironments that repulse immune cells or render them inactive is better understood. Cancer cells produce hypoxic and acidic environments that are immunosuppressive (Huber et al., 2017; Multhoff and Vaupel, 2020; Vito et al., 2020; **Figure 1O**). In particular, cancer cells upregulating carbonic anhydrase IX (CAIX) have been shown to produce both hypoxic and acidic conditions. Such cancer cells are more aggressive, metastatic, and immunosuppressive (Pastorekova and Gillies, 2019). In breast cancer, Lloyd et al. (2016) showed that CAIX-expressing cancer cells predominated at the edges of tumors where immune infiltration was highest.

The anti-predator adaptations of cancer cells that most closely align with those seen in natural predator-prey systems are noxious defenses. Though, here again, cancer cells exploit some of the unique regulatory properties of the immune system designed to minimize injury to self. Antigen expression by a cancer cell stimulates several signaling cascades within tumor-specific activated T-cells including regulatory receptors such as the programmed cell death protein 1 (PD-1) (Pardoll, 2012). As an extra precaution against T-cells killing the wrong cells, PD-1 on the T-cell interacts with its ligand PD-L1 on the surface of normal cells. Frequently, across multiple cancer types and across patients, cancer cells independently evolve to upregulate PD-L1, covering their cell surfaces with these transmembrane ligand binding proteins PD-L1 (Dong et al., 1999; Atefi et al., 2014). When a T-cell encounters the cancer and the PD-L1 binds to the T-cell's PD-1 protein a "no killing" command ensues. In response, the T-cell may leave the cancer alone, or, in terms of inducing injury, the T-cell may cease to divide, deactivate, or even undergo apoptosis (Butte et al., 2007; Francisco et al., 2009). This form of immune checkpoint adaptation by the cancer cells also manifests in other death inducing FAS-FAS ligand binding, CD47, and HLA-G (Pettersen, 2000; Horton et al., 2018; Zhu et al., 2019). When the cancer cells present receptors that induce death or deactivation of the T-cells, they possess a noxious defense.

The noxious anti-predator repertoire of cancer cells includes releasing chemicals that deactivate or induce apoptosis in cytotoxic cells (e.g., release of NK-cell ligands). The secretion of interferons and TNFα by infiltrating lymphocytes amplifies the immune response by attracting cytotoxic T-cells, NK cells, and macrophages. However, experimental data from both mice (Spranger et al., 2013) and humans (Rooney et al., 2015) show that interferons also induce the expression of indolamine 2,3 dioxygenase (IDO). Increased level of IDO in the tumor microenvironment leads to metabolic suppression of the lymphocytes and a reduction of cytotoxic immune cells. Cancer cells also evolve to release extracellular vesicles (EV) that can

contain immune checkpoints, signaling molecules that attract pro-tumor immune cells such as T-regs and M2 macrophages. These pro-tumor cells suppress the proliferation of cytotoxic immune cells and even co-feed cancer cells. In effect, the cancer cells can evolve adaptions to co-opt and hijack the immune system (Heusinkveld and van Der Burg, 2011; Kareva, 2011). In this ultimate form of noxiousness, the cancer cells call in the "enemy" of their "enemy." Cancer cells have developed indirect defenses very much akin to plants releasing volatile chemicals to attract predatory wasps of the plant's arthropod pests (Halitschke et al., 2008).

# FROM NATURE TO CANCER AND BACK AGAIN

## Looks Can Be Deceiving

We have presented examples of diverse species employing armor, spines, and noxious chemicals as anti-predator adaptations. It is evident from this overview that defenses with the same essential function do not always look similar, and traits that look similar do not always function similarly. One essential defense strategy we have described is specialized morphology that increases predator subduing and/or handling time. In mammals and other animals, this defense typically looks like armor, that is, exceptionally durable integument. In other species, this defense strategy often takes the form of a robust exterior, but sometimes it does not. For example, filamentous morphology in bacteria and cancer increases predator handling time even without the thickening of cell walls (Jürgens et al., 1994; Pernthaler et al., 1996; Hahn et al., 1999).

Spine-like morphologies are widespread across taxa, but the function of these morphologies varies. Some species' spines are clearly dangerous to predators, such as the exaggerated spines of porcupines (**Figure 1F**). In other cases, it is less evident that spine-like morphologies are capable of hurting predators and may instead function primarily to increase predator subduing or handling time. For example, the presence of *Daphnia* (zooplankton grazers) triggers *Scenedesmus* phytoplankton form linked-up colonies and develop spines (van Donk and Hessen, 1993; Pančić and Kiørboe, 2018). This morphology reduces predation by small *Daphnia*, suggesting that the function of the spines is to make the *Scenedesmus* colonies larger and thereby more difficult to handle. This echoes the hypothesis of Price et al. (2015) that fish spines evolved to thwart gape-limited piscivores. Spine-like morphologies in cancer may serve multiple purposes including movement, degradation of extra-cellular matrix, and to keep cytotoxic T-cells at bay. These functions need not be mutually exclusive. The protuberances of PD-L1 transmembrane proteins in cancer cells may be more akin functionally to the rays of lionfish (*Pterois* spp.) (**Figure 1G**). While cancer cells and lionfish face very different predators, each can result in the predator's injury or death. The lesson from this overview is that defenses functioning like armor and spines (terms based on mammalian examples) are widespread across nature, but the function of morphological defenses cannot be assumed from their appearance alone.

## Community Composition

The benefits of armor, spines, and noxious defenses will vary according to the frequency of defended prey in the system as well as the type of predator. If all prey of an ecosystem had 100% effective defenses, then their predators would starve and there would be none. Similarly, if cancer cells within their tumor exerted 100% effective immunosuppression, there would be no activated immune response. But, in the absence of any predators or immune response, natural selection would favor prey and cancer cells without spines, armor, or noxiousness. Conversely, if prey were poorly defended or defenses were rare, then the population size of predators would be abundant, thus favoring more highly defended prey. Predator-prey systems in nature should equilibrate on a mix of vulnerable and defended prey; or prey with some intermediate level of defense. For example, though North American porcupines (*Erethizon dorsatum*) are robustly defended by barbed spines, their defense is not so robust to be able to thwart specialist predators such as fishers (*Pekania pennanti*) (DeWitt et al., 2019).

Because vulnerable prey support the predators that select for armored, spiny, and noxious prey, and defended prey support few predators, thus favoring undefended prey, we imagine a mix of vulnerable and defended prey species in most natural ecosystems. In particular, defenses such as armor, spines, and noxiousness seem to be favored when the prey experience high encounter rates with predators and when they need to maintain conspicuous feeding activities in the face of these threats. Cancer cells almost always live in microenvironments with cytotoxic threats from the innate and adaptive immune system. They cannot really flee nor hide (entirely escape detection), so armor-like, spine-like, and noxious defenses should be the norm. While camouflage, as such, is not an option because immune cells will encounter just about all cells in the tumor, there can be a form of serendipitous or perhaps adaptive protection by having a ring of cancer-associated fibroblasts form a physical enclosure of cells and extracellular matrix that blocks off immune cells (Hilmi et al., 2020).

For prey with armor, spines, and noxious defenses that are targeted by immune systems, it is less straightforward to predict how the frequency of defended prey in the system will affect the effectiveness of each defense. Soldier-caste-type predators will not avoid attacking defended prey just because easier prey are available. A lot will depend on the feedback between the frequency of defended cells and the degree of anti-tumor immune infiltration. If, for instance, defense comes in the form of not presenting antigens, then cytotoxic T-cells will largely ignore them and be more available to attack antigen presenting cancer cells. If all other cancer cells are defended, then it behooves a cancer cell to conform. In this case, all "armored" cancer cells become an evolutionarily stable strategy (ESS). If the cancer's defense comes in the form of directly or indirectly causing the inactivation or death of cytotoxic immune cells, then these cancer cells provide a "public good" that may promote freeloading at the ESS. A cancer cell surrounded by noxiously defended cancer cells may need no defense; while a cancer cell surrounded by undefended ones may do best by being noxious. With noxious defenses, the ESS community may be a mix of defended and

less defended cancer cells. The coexistence of immune-evasive and immune-susceptible cancer cell types is an interesting and important avenue for research.

## Aposematism and Mimicry

Aposematism, or warning signaling, is widespread among animals with noxious defenses (Berenbaum, 1995; Stankowich et al., 2011; Wang et al., 2018b) and dangerous spines (Inbar and Lev-Yadun, 2005). When the prey can induce unacceptable acute or chronic injury, the interests of the prey and predator become aligned: the prey does not want to be attacked and the predator does not want to risk injury. Aposematic signals are not inherently deterring but are so because they are paired with dangerous (noxious and/or spiny) defenses. Likely for this reason, aposematic signals are associated with slow mobility, such as the slow and non-evasive flight styles of noxious butterflies (Srygley, 1994). Since aposematic prey have dangerous defenses, they do not need to flee as a primary defense, so speed is unimportant. Slow movement may also increase the visibility of the warning signals (Srygley, 1994).

Recognition of aposematic signals will increase with instances of Müllerian mimicry, when similar-looking noxious species with shared predators evolve to look even more similar. However, aposematism also opens the door for Batesian mimicry, where a non-dangerous prey dishonestly displays the aposematic signal, taking advantage of predators' reluctance to pursue dangerous prey. The Batesian mimic essentially freeloads off the dangerous prey, reaping the benefits of the defense without incurring the associated costs.

A variety of anti-immune adaptations by cancer cells amount to Batesian mimicry, where the normal cells are the model and the cancer cells are the mimic. For example, cultured melanoma cells that decrease expression of the melanoma differentiation antigen gp100 evade detection by introduced T-cells (Landsberg et al., 2012) by mimicking normal cells. They do this while remaining cancer cells and not by becoming normal cells. At first glance it seems odd to classify this type of mimicry as Batesian. Unlike traditional predators that are working on commission, immune cells are not deterred from pursuing dangerous prey. However, a danger which immune cells do avoid is auto-immunity or self-attack. Cancer cells take advantage of immune cells' reluctance to attack host cells by pretending to be host cells. This might seem like a form of camouflage. It is not. In nature camouflage prevents predator detection, it does not involve the predator finding the prey and deciding to pass it by. Cancer cells pretending to be normal cells do not evade immune cell detection, after all the immune cells are contacting normal cells and cancer cells alike to detect the antigens on their surfaces. Rather, what the mimic cancer cells really evade is accurate identification by immune cells. In this sense, it would be useful to interpret disguised cancer cells as Batesian mimicry. They do not imitate dangerous prey, but they imitate the thing that would be dangerous for immune cells to attack. **Figure 2** outlines these dynamics. One will note that for cancer cells mimicking normal cells, this strategy conforms to Batesian mimicry (mimicking the prey that is dangerous to attack) and armor (increasing predator handling time). Both perspectives may prove useful for future research.

# CONCLUSION

Anti-predator defenses in the form of armor, spines, and noxious chemicals are found widely across taxa in nature. They are also displayed by cancer cells. Prey using these modes of defense can continue activities such as foraging and proliferating even when predators are numerous and nearby, which can offer a distinct advantage over strategies such as fleeing, camouflage, or seeking refuge. Armor-like defenses are those which make prey difficult and time-consuming for predators to subdue and/or handle. Noxious defenses make prey dangerous; they can temporarily or permanently incapacitate predators. Spiny defenses may fall into either or both functional categories. Traditional predators will be effort-deterred and injury-deterred from pursuing prey defended in these ways.

Cancer cells experience incessant predation pressure from the immune system. By the nature of their morphology and environment, cancer cells cannot flee, hide, or remain camouflaged. Armor, spines, and noxious chemicals are thus their primary recourse for defense. These defense modes take considerably different forms in cancer cells than in other taxa, but their essential functions are the same. However, the motives of the predators are quite different in cancer versus natural systems. The cytotoxic cells of the immune system are non-traditional, soldier-caste-type predators which do not work on commission. They are not deterred from pursuing difficult or dangerous prey. Because immune cells carefully avoid attacking normal cells of the whole organism, cancer cells, like Batesian mimics, frequently capitalize on this reticence by mimicking normal cells. Furthermore, the regulatory agents of the immune system can control the deployment, proliferation, and death of cytotoxic immune cells. Cancer cells "hijack" these communications to not only suppress cytotoxic immune cells but to amplify pro-tumor immune cells.

For ecologists, cancer provides replicated worlds for studying the parallel and convergent evolution of anti-predator adaptations within different microenvironments of a tumor (habitat scale in ecology) and separate tumors of the host (biome scale with the same taxa). The same evolutionary ecology can be studied in the same cancer across patients (replaying the tape of life for roughly the same taxa) or different cancers across patients (replaying the tape of life with different taxonomic origins; for instance, colon cancer versus liver cancer patients). Furthermore, cancer research centers often possess technologies and equipment related to cell culturing, cell sorting, molecular analyses, mouse model experiments, and histologies that might be unavailable to ecologists studying natural systems.

For oncologists, empirical and conceptual work on anti-predator adaptations draws attention to the functional role that traits play in deterring predation, not just the form. Most immunotherapies, or cancer therapies in general, take advantage of a molecular target, or in the case of recent advances like CAR T-cell therapy, the actual cancer cells themselves as targetable. In our context, this therapy makes cancer cells distinguishable from normal cells, thus acting to strip away the cancer cells' armor or Batesian mimicry. This can create a bit of tunnel vision where two things are overlooked. First, the entire context of the cancer cells'

adaptations, both to their microenvironment and for immune evasion, might be overlooked. Second, less attention is paid to how the cancer cells might or will evolve therapy resistance and effective countermeasures to the therapy.

CAR T-cell therapy can be highly effective in promoting a complete or partial response in some patients while barely hindering progressive disease in others (Wagner et al., 2020). Majzner and Mackall (2018) note that CAR T-cell therapy failure in solid tumors often results from the cancer cells downregulating or eliminating the T-cell presenting antigen. In response to issues of toxicity, T-cell infiltration into the tumor, downregulation of immunogenicity, and tumor heterogeneity, much research is going into manufacturing safer, more effective, and more applicable CAR T-cell products (Rafiq et al., 2020), which is all well and good. But, little of this work considers the ecological context of the cancer cells, the cost and benefits of their current immune evasion strategies, and the ease or difficulty that they will have in evolving an effective anti-predator response. The same applies to research on the efficacy of other current immunotherapies such as Pembrolizumab (anti PD-1 inhibitor, which works against "spines" and "noxiousness"), Nivolumab (anti PD-1 inhibitor), and Ipilimumab (CTLA-4 blocker, which works against "noxiousness"). Often Nivolumab and Ipilimumab are given together, and any of these immunotherapies may be at times combined with chemotherapy, radiation therapy, and surgery. Such therapies act against the current adaptations of the cancer cells but do not anticipate how the cancer cells will evolve new forms of immune evasion using armor, spines, or noxiousness. How easily can they evolve and at what cost to the cancer cells' performance?

We feel that incorporating the perspectives of this overview can provide insights and direct research into the successes and failures of diverse immunotherapies and, perhaps, suggest novel therapeutic strategies. These concepts may help with anticipating rather than reacting to the cancer's evolution of immunosuppression and resistance to immunotherapies. Just as the cancer cells exploit weaknesses in the immune system, so should the physicians find weaknesses in the current strategies of the cancer cells and anticipate how they will respond to various immunotherapies. If the physician is going to use the immune system for biological control, the therapy regime must be dynamic and change as the cancer cells' anti-predator strategies change. Knowledge from ecology may assist in framing the anti-predator options available to the cancer cells and suggest how to anticipate the kinds of armor, spines, and noxiousness that might occur in response the therapeutic regimens. Most therapies are given until disease progression, at which point the cancer cells have long since evolved countermeasures. With this in mind, physicians can use immunotherapies in a more dynamic fashion to anticipate and steer the cancers' evolution while driving down the population of cancer cells (Cunningham et al., 2012).

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

JP and JB developed the concept and guided the collaboration. JP, MM, CW, and JB made major writing contributions. KF, EP, AP, KC, and SA contributed to writing. JP and KF designed the figures. MM, EP, KC, and JB contributed to the figures. JP formatted the manuscript. All authors conducted the literature research and contributed comments and edits to the manuscript.

## FUNDING

JB acknowledges funding from NIH/NCI 1U54CA193489-01A1, "Cancer as a Complex Adaptive System," and NIH/NCI U54 Supplement, "The tumor-host evolutionary arms race."

## REFERENCES

Alterman, L. (1995). "Toxins and toothcombs: potential allospecific chemical defenses in *Nycticebus* and *Perodicticus*," in *Creatures of the Dark: The Nocturnal Prosimians*, eds L. Alterman, G. A. Doyle, and M. K. Izard (Boston, MA: Springer), 413–424. doi: 10.1007/978-1-4757-2405-9_24

Anichini, A., Perotti, V. E., Sgambelluri, F., and Mortarini, R. (2020). Immune escape mechanisms in non small cell lung cancer. *Cancers (Basel).* 12:3605. doi: 10.3390/cancers12123605

Atefi, M., Avramis, E., Lassen, A., Wong, D. J. L., Robert, L., Foulad, D., et al. (2014). Effects of MAPK and PI3K pathways on PD-L1 expression in melanoma. *Clin. Cancer Res.* 20, 3446–3457. doi: 10.1158/1078-0432.ccr-13-2797

Augoff, K., Hryniewicz-Jankowska, A., and Tabola, R. (2020). Invadopodia: clearing the way for cancer cell invasion. *Ann. Transl. Med.* 8:902. doi: 10.21037/atm.2020.02.157

Baty, A. M., Eastburn, C. C., Techkarnjanaruk, S., Goodman, A. E., and Geesey, G. G. (2000). Spatial and temporal variations in chitinolytic gene expression and bacterial biomass production during chitin degradation. *Appl. Environ. Microbiol.* 66, 3574–3585. doi: 10.1128/aem.66.8.3574-3585.2000

Beatty, G. L., and Gladney, W. L. (2015). Immune escape mechanisms as a guide for cancer immunotherapy. *Clin. Cancer Res.* 21, 687–692. doi: 10.1158/1078-0432.ccr-14-1860

Berenbaum, M. R. (1995). Aposematism and mimicry in caterpillars. *J. Lepid. Soc.* 49, 386–396.

Blank, C. U., Haanen, J. B., Ribas, A., and Schumacher, T. N. (2016). The "cancer immunogram". *Science* 352, 658–660. doi: 10.1126/science.aaf2834

Blum, M. (1981). *Chemical Defenses of Arthropods*. New York, NY: Academic Press.

Breed, M. D., and Bennett, B. (1987). "Kin recognition in highly eusocial insects," in *Kin Recognition in Animals*, eds D. J. C. Fletcher and C. D. Michener (New York, NY: John Wiley & Sons), 243–285.

Brown, J. S. (2016). Why Darwin would have loved evolutionary game theory. *Proc. R. Soc. Lond. B Biol. Sci.* 283:20160847. doi: 10.1098/rspb.2016.0847

Brown, J. S., and Kotler, B. P. (2004). Hazardous duty pay and the foraging cost of predation. *Ecol. Lett.* 7, 999–1014. doi: 10.1111/j.1461-0248.2004.00661.x

Burnet, M. (1957). Cancer: a biological approach: I. The processes of control. *Br. Med. J.* 1, 779–786. doi: 10.1136/bmj.1.5022.779

Butte, M. J., Keir, M. E., Phamduy, T. B., Sharpe, A. H., and Freeman, G. J. (2007). Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory

molecule to inhibit T cell responses. *Immunity* 27, 111–122. doi: 10.1016/j.immuni.2007.05.016

Chandra, J., McCormick, T. S., Imamura, Y., Mukherjee, P. K., and Ghannoum, M. A. (2007). Interaction of *Candida albicans* with adherent human peripheral blood mononuclear cells increases *C. albicans* biofilm formation and results in differential expression of pro-and anti-inflammatory cytokines. *Infect. Immun.* 75, 2612–2620. doi: 10.1128/iai.01841-06

Coley, W. B. (1891). II. Contribution to the knowledge of sarcoma. *Ann. Surg.* 14, 199–220. doi: 10.1097/00000658-189112000-00015

Corthay, A. (2014). Does the immune system naturally protect against cancer? *Front. Immunol.* 5:197. doi: 10.3389/fimmu.2014.00197

Crosland, M. W. J. (1990). Variation in ant aggression and kin discrimination ability within and between colonies. *J. Insect Behav.* 3, 359–379. doi: 10.1007/bf01052114

Cunningham, J. J., Gatenby, R. A., and Brown, J. S. (2012). Evolutionary dynamics in cancer therapy. *Mol. Phylogenet.* 8, 2094–2100. doi: 10.1021/mp2002279. Evolutionary

Cuyler, W. K. (1924). Observations on the habits of the striped skunk (*Mephitis mesomelas varians*). *J. Mammal.* 5, 180–189. doi: 10.2307/1373285

Dahl, E. (2019). *Dinophysis Bloom in Norway. US National Office Harmful Algal Bloom. Woods Hole Oceanographic Institution*. Available online at: https://hab.whoi.edu/regions-resources/gallery/photos-blooms/ (accessed March 13, 2021).

DavidpBowman (2014). *Trichocyst. Wikimedia Commons, CC BY-SA 4.0*. Available online at: https://commons.wikimedia.org/wiki/File:Trichocyst.jpg (accessed March 13, 2021).

Davies, R. G. (1988). "Insect structure and function," in *Outlines of Entomology*, ed. R. G. Davies (Dordrecht: Springer), 7–96. doi: 10.1007/978-94-009-1189-5_2

de Guillebon, E., Dardenne, A., Saldmann, A., Séguier, S., Tran, T., Paolini, L., et al. (2020). Beyond the concept of cold and hot tumors for the development of novel predictive biomarkers and the rational design of immunotherapy combination. *Int. J. Cancer* 147, 1509–1518. doi: 10.1002/ijc.32889

de Pillis, L. G., Radunskaya, A. E., and Wiseman, C. L. (2005). A validated mathematical model of cell-mediated immune response to tumor growth. *Cancer Res.* 65, 7950–7958. doi: 10.1158/0008-5472.can-05-0564

Demaria, O., Cornen, S., Daëron, M., Morel, Y., Medzhitov, R., and Vivier, E. (2019). Harnessing innate immunity in cancer therapy. *Nature* 574, 45–56. doi: 10.1038/s41586-019-1593-5

DeWitt, P. D., Visscher, D. R., Schuler, M. S., and Thiel, R. P. (2019). Predation risks suppress lifetime fitness in a wild mammal. *Oikos* 128, 790–797. doi: 10.1111/oik.05935

Dong, H., Zhu, G., Tamada, K., and Chen, L. (1999). B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat. Med.* 5, 1365–1369. doi: 10.1038/70932

Dullens, H. F. J., Van Der Tol, M. W. M., De Weger, R. A., and Den Otter, W. (1986). A survey of some formal models in tumor immunology. *Cancer Immunol. Immunother.* 23, 159–164. doi: 10.1007/bf00205644

Dunn, G. P., Old, L. J., and Schreiber, R. D. (2004). The three Es of cancer immunoediting. *Annu. Rev. Immunol.* 22, 329–360. doi: 10.1146/annurev.immunol.22.012703.104803

Eftimie, R., Gillard, J. J., and Cantrell, D. A. (2016). Mathematical models for immunology: current state of the art and future research directions. *Bull. Math. Biol.* 78, 2091–2134. doi: 10.1007/s11538-016-0214-9

Eisner, T. (1970). "Chemical defense against predation in arthropods," in *Chemical Ecology*, eds J. B. Simeone and E. Sondheimer (Cambridge, MA: Academic Press), 157–217. doi: 10.1016/b978-0-12-654750-4.50014-1

Eisner, T., Eisner, M., and Siegler, M. (2005). *Secret Weapons: Defenses of Insects, Spiders, Scorpions, and Other Many-Legged Creatures*. Cambridge, MA: Harvard University Press.

Elbroch, L. M., Hoogesteijn, R., and Quigley, H. (2016). Cougars (*Puma concolor*) killed by North American porcupines (*Erethizon dorsatum*). *Can. Field Nat.* 130, 53–55. doi: 10.22621/cfn.v130i1.1793

Esfahani, K., Roudaia, L., Buhlaiga, N., Del Rincon, S. V., Papneja, N., and Miller, W. H. Jr. (2020). A review of cancer immunotherapy: from the past, to the present, to the future. *Curr. Oncol.* 27:S87.

Fishwild, T. G., and Gamboa, G. J. (1992). Colony defence against conspecifics: caste-specific differences in kin recognition by paper wasps, *Polistes fuscatus*. *Anim. Behav.* 43, 95–102. doi: 10.1016/s0003-3472(05)80075-2

Flenner, I. D. A., Olne, K., Suhling, F., and Sahlén, G. (2009). Predator-induced spine length and exocuticle thickness in *Leucorrhinia dubia* (Insecta: Odonata): a simple physiological trade-off? *Ecol. Entomol.* 34, 735–740. doi: 10.1111/j.1365-2311.2009.01129.x

Forbes, L. S. (1989). Prey defences and predator handling behaviour: the dangerous prey hypothesis. *Oikos* 55, 155–158. doi: 10.2307/3565418

Forti, L. R., da Silva, R. M., Manzani, P. R., and Toledo, L. F. (2018). Snakes vs. porcupines: when preys leave mortal injuries. *Herpetol. Notes* 11, 715–718.

Fouad, Y. A., and Aanei, C. (2017). Revisiting the hallmarks of cancer. *Am. J. Cancer Res.* 7, 1016–1036.

Fowler, S. W., and Fisher, N. S. (1983). Viability of marine phytoplankton in zooplankton fecal pellets. *Deep Sea Res. Part A. Oceanogr. Res. Pap.* 30, 963–969. doi: 10.1016/0198-0149(83)90051-1

Fox, C. (2001). *Cancer Cells, AV-8711-3170. National Cancer Institute*. Available online at: https://visualsonline.cancer.gov/details.cfm?imageid=2306 (accessed March 13, 2021).

Francisco, L. M., Salinas, V. H., Brown, K. E., Vanguri, V. K., Freeman, G. J., Kuchroo, V. K., et al. (2009). PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J. Exp. Med.* 206, 3015–3029. doi: 10.1084/jem.20090847

Fridman, W. H. (2018). From cancer immune surveillance to cancer immunoediting: birth of modern immuno-oncology. *J. Immunol.* 201, 825–826. doi: 10.4049/jimmunol.1800827

Gall, B. G., Spivey, K. L., Chapman, T. L., Delph, R. J., Brodie, E. D. Jr., and Wilson, J. S. (2018). The indestructible insect: velvet ants from across the United States avoid predation by representatives from all major tetrapod clades. *Ecol. Evol.* 8, 5852–5862. doi: 10.1002/ece3.4123

Gartner, J. (2021). *Lymphocytes and Cancer Cell, Image 488635633*. New York, NY: Getty Images Science Photo Library.

Gatenby, R. A., and Brown, J. (2017). Mutations, evolution and the central role of a self-defined fitness function in the initiation and progression of cancer. *Biochim. Biophys. Acta Rev. Cancer* 1867, 162–166. doi: 10.1016/j.bbcan.2017.03.005

Gatenby, R. A., Avdieiev, S., Tsai, K. Y., and Brown, J. S. (2020). Integrating genetic and nongenetic drivers of somatic evolution during carcinogenesis: the biplane model. *Evol. Appl.* 13, 1651–1659. doi: 10.1111/eva.12973

Ge, Y., Zhang, L., Qin, Z., Wang, Y., Liu, P., Tan, S., et al. (2019). Different predation capacities and mechanisms of *Harmonia axyridis* (Coleoptera: Coccinellidae) on two morphotypes of pear psylla *Cacopsylla chinensis* (Hemiptera: Psyllidae). *PLoS One* 14:e0215834. doi: 10.1371/journal.pone.0215834

Greenwood, A. K., Peichel, C. L., and Zottoli, S. J. (2010). Distinct startle responses are associated with neuroanatomical differences in pufferfishes. *J. Exp. Biol.* 213, 613–620. doi: 10.1242/jeb.037085

Grivennikov, S. I., Greten, F. R., and Karin, M. (2010). Immunity, inflammation, and cancer. *Cell* 140, 883–899.

Grønning, J., and Kiørboe, T. (2020). Diatom defence: grazer induction and cost of shell-thickening. *Funct. Ecol.* 34, 1790–1801. doi: 10.1111/1365-2435.13635

Guirguis, R., Margulies, I., Taraboletti, G., Schiffmann, E., and Liotta, L. (1987). Cytokine-induced pseudopodial protrusion is coupled to tumour cell migration. *Nature* 329, 261–263. doi: 10.1038/329261a0

Hahn, M. W., Moore, E. R. B., and Höfle, M. G. (1999). Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. *Appl. Environ. Microbiol.* 65, 25–35. doi: 10.1128/aem.65.1.25-35.1999

Haim, A., Van Aarde, R. J., and Skinner, J. D. (1990). Metabolism and thermoregulation in the Cape porcupine, *Hystrix africaeaustralis*. *Physiol. Zool.* 63, 795–802. doi: 10.1086/physzool.63.4.30158177

Halitschke, R., Stenberg, J. A., Kessler, D., Kessler, A., and Baldwin, I. T. (2008). Shared signals–'alarm calls' from plants increase apparency to herbivores and their enemies in nature. *Ecol. Lett.* 11, 24–34.

Hall-Stoodley, L., Costerton, J. W., and Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.* 2, 95–108. doi: 10.1038/nrmicro821

Hamm, C. E., Merkel, R., Springer, O., Jurkojc, P., Maier, C., Prechtel, K., et al. (2003). Architecture and material properties of diatom shells provide effective mechanical protection. *Nature* 421, 841–843. doi: 10.1038/nature01416

Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* 100, 57–70.

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Harris, R. J., and Jenner, R. A. (2019). Evolutionary ecology of fish venom: adaptations and consequences of evolving a venom system. *Toxins (Basel).* 11, 60. doi: 10.3390/toxins11020060

Harvey, E. L., Bidle, K. D., and Johnson, M. D. (2015). Consequences of strain variability and calcification in *Emiliania huxleyi* on microzooplankton grazing. *J. Plankton Res.* 37, 1137–1148.

Hedgcock, C. (2013). *Bombardier beetle, Brachinus elongatulus (Chaudoir)*. Available online at: https://charleshedgcock.photoshelter.com/gallery-image/Charismatic-Microfauna/G0000rqy5jilPT5w/I00008wHp8dL3NhY (accessed June 7, 2021).

Hertz, J. C. (2007). *Velvet Ants, Mutillidae (Insecta: Hymenoptera): EENY-378*. Gainesville, FL: Florida Cooperative Extension Service Institute of Food and Agricultural Sciences University of Florida.

Heusinkveld, M., and van Der Burg, S. H. (2011). Identification and manipulation of tumor associated macrophages in human cancers. *J. Transl. Med.* 9, 1–14.

Hill, R. V. (2006). Comparative anatomy and histology of xenarthran osteoderms. *J. Morphol.* 267, 1441–1460. doi: 10.1002/jmor.10490

Hilmi, M., Nicolle, R., Bousquet, C., and Neuzillet, C. (2020). Cancer-associated fibroblasts: accomplices in the tumor immune evasion. *Cancers (Basel).* 12:2969. doi: 10.3390/cancers12102969

Hirschfeld, J. (2014). Dynamic interactions of neutrophils and biofilms. *J. Oral Microbiol.* 6:26102. doi: 10.3402/jom.v6.26102

Hodge, J. R., Alim, C., Bertrand, N. G., Lee, W., Price, S. A., Tran, B., et al. (2018). Ecology shapes the evolutionary trade-off between predator avoidance and defence in coral reef butterflyfishes. *Ecol. Lett.* 21, 1033–1042. doi: 10.1111/ele.12969

Horton, B. L., Williams, J. B., Cabanov, A., Spranger, S., and Gajewski, T. F. (2018). Intratumoral CD8+ T-cell apoptosis is a major component of T-cell dysfunction and impedes antitumor immunity. *Cancer Immunol. Res.* 6, 14–24. doi: 10.1158/2326-6066.cir-17-0249

Houghton, A. N. (1994). Cancer antigens: immune recognition of self and altered self. *J. Exp. Med.* 180, 1–4. doi: 10.1084/jem.180.1.1

Huber, V., Camisaschi, C., Berzi, A., Ferro, S., Lugini, L., Triulzi, T., et al. (2017). "Cancer acidity: an ultimate frontier of tumor immune escape and a novel target of immunomodulation," in *Seminars in Cancer Biology*, eds S. Harguindey and S. J. Reshkin (Amsterdam: Elsevier), 74–89. doi: 10.1016/j.semcancer.2017.03.001

Inbar, M., and Lev-Yadun, S. (2005). Conspicuous and aposematic spines in the animal kingdom. *Naturwissenschaften* 92, 170–172. doi: 10.1007/s00114-005-0608-2

Jansen, R., Sodeinde, O., Soewu, D., Pietersen, D. W., Alempijevic, D., and Ingram, D. J. (2020). "White-bellied pangolin *Phataginus tricuspis* (Rafinesque, 1820)," in *Pangolins*, eds D. W. S. Challender, H. C. Nash, and C. Waterman (Amsterdam: Elsevier), 139–156. doi: 10.1016/b978-0-12-815507-3.00009-5

Jensen, E. T., Kharazmi, A., Høiby, N., and Costeron, J. W. (1992). Some bacterial parameters influencing the neutrophil oxidative burst response to *Pseudomonas aeruginosa* biofilms. *APMIS* 100, 727–733. doi: 10.1111/j.1699-0463.1992.tb03991.x

Jesaitis, A. J., Franklin, M. J., Berglund, D., Sasaki, M., Lord, C. I., Bleazard, J. B., et al. (2003). Compromised host defense on *Pseudomonas aeruginosa* biofilms: characterization of neutrophil and biofilm interactions. *J. Immunol.* 171, 4329–4339. doi: 10.4049/jimmunol.171.8.4329

Jeschke, J. M. (2006). Density-dependent effects of prey defenses and predator offenses. *J. Theor. Biol.* 242, 900–907. doi: 10.1016/j.jtbi.2006.05.017

Jürgens, K., Arndt, H., and Rothhaupt, K. O. (1994). Zooplankton-mediated changes of bacterial community structure. *Microb. Ecol.* 27, 27–42.

Kageyama, A., and Sugiura, S. (2016). Caterpillar hairs as an anti-parasitoid defence. *Sci. Nat.* 103, 86.

Kareva, I. (2011). What can ecology teach us about cancer? *Transl. Oncol.* 4, 266–270. doi: 10.1593/tlo.11154

Kareva, I., Berezovskaya, F., and Castillo-Chavez, C. (2010). Myeloid cells in tumour–immune interactions. *J. Biol. Dyn.* 4, 315–327. doi: 10.1080/17513750903261281

Katzner, T., Miller, T. A., Rodrigue, J., and Shaffer, S. (2015). A most dangerous game: death and injury to birds from porcupine quills. *Wilson J. Ornithol.* 127, 102–108. doi: 10.1676/14-066.1

Kaur, G., and Ahmad, N. (2014). On study of immune response to tumor cells in prey-predator system. *Int. Sch. Res. Notices* 2014:346597.

Keck, W. (2011). *Skunk about to Spray. National Park Service*. Available online at: https://commons.wikimedia.org/wiki/File:Skunk_about_to_spray.jpg (accessed March 13, 2021).

Kerbis Peterhans, J. C., Celesia, G. G., and Gnoske, T. P. (2019). Lion-porcupine interactions in Africa, including impacts on lion predatory behavior. *J. East African Nat. Hist.* 108, 1–15. doi: 10.2982/028.108.0101

Kharazmi, A. (1991). Mechanisms involved in the evasion of the host defence by *Pseudomonas aeruginosa*. *Immunol. Lett.* 30, 201–205. doi: 10.1016/0165-2478(91)90026-7

Khong, H. T., and Restifo, N. P. (2002). Natural selection of tumor variants in the generation of "tumor escape" phenotypes. *Nat. Immunol.* 3, 999–1005. doi: 10.1038/ni1102-999

Kingdon, J., Agwanda, B., Kinnaird, M., O'Brien, T., Holland, C., Gheysens, T., et al. (2012). A poisonous surprise under the coat of the African crested rat. *Proc. R. Soc. Lond. B Biol. Sci.* 279, 675–680. doi: 10.1098/rspb.2011.1169

Klompmaker, A. A., Kelley, P. H., Chattopadhyay, D., Clements, J. C., Huntley, J. W., and Kowalewski, M. (2019). Predation in the marine fossil record: studies, data, recognition, environmental factors, and behavior. *Earth Sci. Rev.* 194, 472–520. doi: 10.1016/j.earscirev.2019.02.020

Knoll, G., Haacke-Bell, B., and Plattner, H. (1991). Local trichocyst exocytosis provides an efficient escape mechanism for *Paramecium* cells. *Eur. J. Protistol.* 27, 381–385. doi: 10.1016/S0932-4739(11)80256-7

Koebel, C. M., Vermi, W., Swann, J. B., Zerafa, N., Rodig, S. J., Old, L. J., et al. (2007). Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* 450, 903–907. doi: 10.1038/nature06309

Kolb, A., and Strom, S. (2013). An inducible antipredatory defense in haploid cells of the marine microalga *Emiliania huxleyi* (Prymnesiophyceae). *Limnol. Oceanogr.* 58, 932–944. doi: 10.4319/lo.2013.58.3.0932

Künzler, M. (2018). How fungi defend themselves against microbial competitors and animal predators. *PLoS Pathog.* 14:e1007184. doi: 10.1371/journal.ppat.1007184

Landsberg, J., Kohlmeyer, J., Renn, M., Bald, T., Rogava, M., Cron, M., et al. (2012). Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. *Nature* 490, 412–416. doi: 10.1038/nature11538

Lazzeri, L., Senini, C., and Mori, E. (2020). Interspecific aggressions between crested porcupines and roe deer. *Animals* 10:623. doi: 10.3390/ani10040623

Lee, C.-H., Yelensky, R., Jooss, K., and Chan, T. A. (2018). Update on tumor neoantigens and their utility: why it is good to be different. *Trends Immunol.* 39, 536–548. doi: 10.1016/j.it.2018.04.005

Leid, J. G. (2009). Bacterial biofilms resist key host defenses. *Microbe* 4, 66–70.

Lepczyk, C. A., Mertig, A. G., and Liu, J. (2004). Landowners and cat predation across rural-to-urban landscapes. *Biol. Conserv.* 115, 191–201. doi: 10.1016/s0006-3207(03)00107-1

Lima, S. L., and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68, 619–640. doi: 10.1139/z90-092

Lindquist, N. (2002). Chemical defense of early life stages of benthic marine invertebrates. *J. Chem. Ecol.* 28, 1987–2000. doi: 10.1023/A:1020745810968

Lindsay, S. (2019). *Placoid Scales. Austrailian Museum*. Available online at: https://australian.museum/learn/animals/fishes/placoid-scales/#main (accessed March 13, 2021).

Lloyd, M. C., Cunningham, J. J., Bui, M. M., Gillies, R. J., Brown, J. S., and Gatenby, R. A. (2016). Darwinian dynamics of intratumoral heterogeneity: not solely random mutations but also variable environmental selection forces. *Cancer Res.* 76, 3136–3144. doi: 10.1158/0008-5472.can-15-2962

Lovegrove, B. (2000). The zoogeography of mammalian basal metabolic rate. *Am. Nat.* 156, 201–219. doi: 10.1086/303383

Lovegrove, B. G. (2001). The evolution of body armor in mammals: plantigrade constraints of large body size. *Evolution (N. Y.)* 55, 1464–1473. doi: 10.1111/j.0014-3820.2001.tb00666.x

Lürling, M., De Lange, H. J., and Van Donk, E. (1997). Changes in food quality of the green alga *Scenedesmus* induced by *Daphnia* infochemicals: biochemical composition and morphology. *Freshw. Biol.* 38, 619–628. doi: 10.1046/j.1365-2427.1997.00225.x

Ma, H., Krock, B., Tillmann, U., and Cembella, A. (2009). Preliminary characterization of extracellular allelochemicals of the toxic marine dinoflagellate *Alexandrium tamarense* using a *Rhodomonas salina* bioassay. *Mar. Drugs* 7, 497–522. doi: 10.3390/md7040497

Maia-Silva, C., Hrncir, M., Koedam, D., Machado, R. J. P., and Imperatriz-Fonseca, V. L. (2013). Out with the garbage: the parasitic strategy of the mantisfly *Plega hagenella* mass-infesting colonies of the eusocial bee *Melipona subnitida* in northeastern Brazil. *Naturwissenschaften* 100, 101–105. doi: 10.1007/s00114-012-0994-1

Majzner, R. G., and Mackall, C. L. (2018). Tumor antigen escape from CAR T-cell therapy. *Cancer Discov.* 8, 1219–1226. doi: 10.1158/2159-8290.cd-18-0442

Maley, C. C., Aktipis, A., Graham, T. A., Sottoriva, A., Boddy, A. M., Janiszewska, M., et al. (2017). Classifying the evolutionary and ecological features of neoplasms. *Nat. Rev. Cancer* 17:605. doi: 10.1038/nrc.2017.69

Materialscientist (2009). *Gypsy Moth Caterpillar. Wikimedia Commons, CC BY-SA 3.0*. Available online at: https://commons.wikimedia.org/wiki/File:Gypsy_moth_caterpillar.JPG (accessed March 13, 2021).

Mathis, A., and Chivers, D. P. (2003). Overriding the oddity effect in mixed-species aggregations: group choice by armored and nonarmored prey. *Behav. Ecol.* 14, 334–339. doi: 10.1093/beheco/14.3.334

Matz, C., and Kjelleberg, S. (2005). Off the hook–how bacteria survive protozoan grazing. *Trends Microbiol.* 13, 302–307. doi: 10.1016/j.tim.2005.05.009

Matz, C., Deines, P., and Jürgens, K. (2002). Phenotypic variation in *Pseudomonas* sp. CM10 determines microcolony formation and survival under protozoan grazing. *FEMS Microbiol. Ecol.* 39, 57–65. doi: 10.1111/j.1574-6941.2002.tb00906.x

Matz, C., Webb, J. S., Schupp, P. J., Phang, S. Y., Penesyan, A., Egan, S., et al. (2008). Marine biofilm bacteria evade eukaryotic predation by targeted chemical defense. *PLoS One* 3:e2744. doi: 10.1371/journal.pone.0002744

McLean, E. B., and Godin, J.-G. J. (1989). Distance to cover and fleeing from predators in fish with different. *Oikos* 55, 281–290. doi: 10.2307/3565586

McLean, S. (2014). Scent glands of the common brushtail possum (*Trichosurus vulpecula*). *New Zeal. J. Zool.* 41, 193–202. doi: 10.1080/03014223.2014.899506

McNab, B. K. (1984). Physiological convergence amongst ant-eating and termite-eating mammals. *J. Zool. Lond.* 203, 485–510. doi: 10.1111/j.1469-7998.1984.tb02345.x

McNab, B. K. (2008). An analysis of the factors that influence the level and scaling of mammalian BMR. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 151, 5–28. doi: 10.1016/j.cbpa.2008.05.008

McNamara, J. M., and Houston, A. I. (1987). Starvation and predation as factors limiting population size. *Ecology* 68, 1515–1519. doi: 10.2307/1939235

Mengak, M. T. (2005). *Nine-Banded Armadillo (Dasypus novemcinctus*): *Natural History Publication Series 4*. Athens, GA: Universit of Georgia Warnell School of Forestry & Natural Resources.

Mergler, C. J., and Gall, B. G. (2021). Has the indestructible insect met its match? Velvet ants as prey to bufonid toads. *Ethol. Ecol. Evol.* 33, 15–24. doi: 10.1080/03949370.2020.1789747

Merlo, L. M. F., Pepper, J. W., Reid, B. J., and Maley, C. C. (2006). Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer* 6, 924–935. doi: 10.1038/nrc2013

Michener, C. D. (1974). *The Social Behavior of the Bees*. Cambridge, MA: Belknap Press.

Mistry, U. (2019). *Lionfish in India's Andaman Islands. Coral Reef Image Bank*. Available online at: https://www.coralreefimagebank.org/fish (accessed March 13, 2021).

Mohme, M., Riethdorf, S., and Pantel, K. (2017). Circulating and disseminated tumour cells—mechanisms of immune surveillance and escape. *Nat. Rev. Clin. Oncol.* 14:155. doi: 10.1038/nrclinonc.2016.144

Mori, E., Maggini, I., and Menchetti, M. (2014). When quills kill: the defense strategy of the crested porcupine *Hystrix cristata* L., 1758. *Mammalia* 78, 229–234.

Multhoff, G., and Vaupel, P. (2020). "Hypoxia compromises anti-cancer immune responses," in *Oxygen Transport to Tissue XLI. Advances in Experimental Medicine and Biology*, Vol. 1232, eds P. D. Ryu, J. LaManna, D. Harrison, and S. S. Lee (Cham: Springer), 131–143. doi: 10.1007/978-3-030-34461-0_18

Munday, P. L., Schubert, M., Baggio, J. A., Jones, G. P., Caley, M. J., and Grutter, A. S. (2003). Skin toxins and external parasitism of coral-dwelling gobies. *J. Fish Biol.* 62, 976–981. doi: 10.1046/j.1095-8649.2003.00078.x

Nam, A. S., Chaligne, R., and Landau, D. A. (2020). Integrating genetic and non-genetic determinants of cancer evolution by single-cell multi-omics. *Nat. Rev. Genet.* 22, 3–18. doi: 10.1038/s41576-020-0265-5

NCI (2021). *NCI Dictionaries. National Cancer Institute*. Available online at: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cigarette (accessed May 17, 2021).

Nekaris, K. A.-I., Moore, R. S., Rode, E. J., and Fry, B. G. (2013). Mad, bad and dangerous to know: the biochemistry, ecology and evolution of slow loris venom. *J. Venom. Anim. Toxins Incl. Trop. Dis.* 19:21. doi: 10.1186/1678-9199-19-21

Nekaris, K., Weldon, A., Imron, M. A., Maynard, K. Q., Nijman, V., Poindexter, S. A., et al. (2019). Venom in furs: facial masks as aposematic signals in a venomous mammal. *Toxins (Basel).* 11:93. doi: 10.3390/toxins11020093

Nilsson, P. A., and Brönmark, C. (2000). Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. *Oikos* 88, 539–546. doi: 10.1034/j.1600-0706.2000.880310.x

Nilsson, P. A., Brönmark, C., and Pettersson, L. B. (1995). Benefits of a predtor-induced morphology in crucian carp. *Oecologia* 104, 291–296. doi: 10.1007/bf00328363

O'Riain, M. J., and Jarvis, J. U. M. (1997). Colony member recognition and xenophobia in the naked mole-rat. *Anim. Behav.* 53, 487–498. doi: 10.1006/anbe.1996.0299

Oiseth, S. J., and Aziz, M. S. (2017). Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. *J. Cancer Metastasis Treat.* 3, 250–261. doi: 10.20517/2394-4722.2017.41

Pančić, M., and Kiørboe, T. (2018). Phytoplankton defence mechanisms: traits and trade-offs. *Biol. Rev.* 93, 1269–1303. doi: 10.1111/brv.12395

Pandya, P. H., Murray, M. E., Pollok, K. E., and Renbarger, J. L. (2016). The immune system in cancer pathogenesis: potential therapeutic approaches. *J. Immunol. Res.* 2016:4273943.

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12, 252–264. doi: 10.1038/nrc3239

Pastorekova, S., and Gillies, R. J. (2019). The role of carbonic anhydrase IX in cancer development: Links to hypoxia, acidosis, and beyond. *Cancer Metastasis Rev.* 38, 65–77. doi: 10.1007/s10555-019-09799-0

Pemberton, C. (2009). *Velvet Ant. Wikimedia Commons, CC BY-SA 3.0*. Available online at: https://commons.wikimedia.org/wiki/File:Velvet_Ant.jpg (accessed March 13, 2021).

Pernthaler, J., Sattler, B., Simek, K., Schwarzenbacher, A., and Psenner, R. (1996). Top-down effects on the size-biomass distribution of a freshwater bacterioplankton community. *Aquat. Microb. Ecol.* 10, 255–263. doi: 10.3354/ame010255

Pettersen, R. D. (2000). CD47 and death signaling in the immune system. *Apoptosis* 5, 299–306.

Philip, M., and Schietinger, A. (2019). Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections. *Curr. Opin. Immunol.* 58, 98–103. doi: 10.1016/j.coi.2019.04.014

Pickett-Heaps, J. D., Carpente, J., and Koutoulis, A. (1994). "Valve and seta (spine) morphogenesis in the centric diatom *Chaetoceros peruvianus* Brightwell," in *The Protistan Cell Surface*, eds R. Wetherbee, J. D. Pickett-Heaps, and R. A. Andersen (Vienna: Springer), 269–282. doi: 10.1007/978-3-7091-9378-5_16

Pienta, K. J., Hammarlund, E. U., Axelrod, R., Amend, S. R., and Brown, J. S. (2020a). Convergent evolution, evolving evolvability, and the origins of lethal cancer. *Mol. Cancer Res.* 18, 801–810. doi: 10.1158/1541-7786.mcr-19-1158

Pienta, K. J., Hammarlund, E. U., Axelrod, R., Brown, J. S., and Amend, S. R. (2020b). Poly-aneuploid cancer cells promote evolvability, generating lethal cancer. *Evol. Appl.* 13, 1626–1634. doi: 10.1111/eva.12929

Pleizier, N., Wilson, A. D. M., Shultz, A. D., and Cooke, S. J. (2015). Puffed and bothered: personality, performance, and the effects of stress on checkered pufferfish. *Physiol. Behav.* 152, 68–78. doi: 10.1016/j.physbeh.2015.09.011

Pondaven, P., Gallinari, M., Chollet, S., Bucciarelli, E., Sarthou, G., Schultes, S., et al. (2007). Grazing-induced changes in cell wall silicification in a marine diatom. *Protist* 158, 21–28. doi: 10.1016/j.protis.2006.09.002

Price, S. A., Friedman, S. T., and Wainwright, P. C. (2015). How predation shaped fish: the impact of fin spines on body form evolution across teleosts. *Proc. R. Soc. Lond. B Biol. Sci.* 282, 20151428. doi: 10.1098/rspb.2015.1428

Quan, H., Yang, W., Schaible, E., Ritchie, R. O., and Meyers, M. A. (2018). Novel defense mechanisms in the armor of the scales of the "living fossil" coelacanth fish. *Adv. Funct. Mater.* 28:1804237. doi: 10.1002/adfm.201804237

Rafiq, S., Hackett, C. S., and Brentjens, R. J. (2020). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat. Rev. Clin. Oncol.* 17, 147–167. doi: 10.1038/s41571-019-0297-y

Raschi, W., and Tabit, C. (1992). Functional aspects of placoid scales: a review and update. *Mar. Freshw. Res.* 43, 123–147. doi: 10.1071/mf9920123

Rhiel, E., Wöhlbrand, L., Rabus, R., and Voget, S. (2018). Candidates of trichocyst matrix proteins of the dinoflagellate *Oxyrrhis marina*. *Protoplasma* 255, 217–230. doi: 10.1007/s00709-017-1148-2

Rice, S. H. (1985). An anti-predator chemical defense of the marine pulmonate gastropod *Trimusculus reticulatus* (Sowerby). *J. Exp. Mar. Bio. Ecol.* 93, 83–89. doi: 10.1016/0022-0981(85)90150-9

Robertson-Tessi, M., El-Kareh, A., and Goriely, A. (2012). A mathematical model of tumor–immune interactions. *J. Theor. Biol.* 294, 56–73.

Roilides, E., Simitsopoulou, M., Katragkou, A., and Walsh, T. J. (2015). How biofilms evade host defenses. *Microb. Biofilms* 3, 287–300. doi: 10.1128/9781555817466.ch14

Rooney, M. S., Shukla, S. A., Wu, C. J., Getz, G., and Hacohen, N. (2015). Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* 160, 48–61. doi: 10.1016/j.cell.2014.12.033

Saito, T. (1985). Resistibility of toxic and nontoxic pufferfish against tetrodotoxin. *Nippon Suisan Gakkaishi* 51:1371. doi: 10.2331/suisan.51.1371

Scherr, T. D., Hanke, M. L., Huang, O., James, D. B. A., Horswill, A. R., Bayles, K. W., et al. (2015). *Staphylococcus aureus* biofilms induce macrophage dysfunction through leukocidin AB and alpha-toxin. *MBio* 6:e01021-15.

Schmidt, J. O. (2016). *The Sting of the Wild*. Baltimore, MD: Johns Hopkins University Press.

Schmidt, J. O., and Blum, M. S. (1977). Adaptations and responses of *Dasymutilla occidentalis* (Hymenoptera: Mutillidae) to predators. *Entomol. Exp. Appl.* 21, 99–111. doi: 10.1111/j.1570-7458.1977.tb02663.x

Seiler, C., van Velzen, E., Neu, T. R., Gaedke, U., Berendonk, T. U., and Weitere, M. (2017). Grazing resistance of bacterial biofilms: a matter of predators' feeding trait. *FEMS Microbiol. Ecol.* 93:fix112. doi: 10.1093/femsec/fix112

Shankaran, V., Ikeda, H., Bruce, A. T., White, J. M., Swanson, P. E., Old, L. J., et al. (2001). IFNγ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 410, 1107–1111. doi: 10.1038/35074122

Sharma, P., Alsharif, S., Fallatah, A., and Chung, B. M. (2019). Intermediate filaments as effectors of cancer development and metastasis: a focus on keratins, vimentin, and nestin. *Cells* 8:497. doi: 10.3390/cells8050497

Smith, B. H., and Breed, M. D. (1995). "The chemical basis for nestmate recognition and mate discrimination in social insects," in *Chemical Ecology of Insects II*, eds R. T. Carde and W. J. Bell (New York, NY: Chapman and Hall), 287–317. doi: 10.1007/978-1-4615-1765-8_8

Smith, W. L., Stern, J. H., Girard, M. G., and Davis, M. P. (2016). Evolution of venomous cartilaginous and ray-finned fishes. *Integr. Comp. Biol.* 56, 950–961. doi: 10.1093/icb/icw070

Smodlaka Tanković, M., Baričević, A., Ivančić, I., Kužat, N., Medić, N., Pustijanac, E., et al. (2018). Insights into the life strategy of the common marine diatom *Chaetoceros peruvianus* Brightwell. *PLoS One* 13:e0203634. doi: 10.1371/journal.pone.0203634

Solinas, G., Germano, G., Mantovani, A., and Allavena, P. (2009). Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. *J. Leukoc. Biol.* 86, 1065–1073. doi: 10.1189/jlb.0609385

Song, J., Ortiz, C., and Boyce, M. C. (2011). Threat-protection mechanics of an armored fish. *J. Mech. Behav. Biomed. Mater.* 4, 699–712. doi: 10.1016/j.jmbbm.2010.11.011

Spranger, S., Spaapen, R. M., Zha, Y., Williams, J., Meng, Y., Ha, T. T., et al. (2013). Up-regulation of PD-L1, IDO, and Tregs in the melanoma tumor microenvironment is driven by CD8+ T cells. *Sci. Transl. Med.* 5:200ra116. doi: 10.1126/scitranslmed.3006504

Srygley, R. B. (1994). ). Locomotor mimicry in butterflies? The associations of positions of centres of mass among groups of mimetic, unprofitable prey. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 343, 145–155. doi: 10.1098/rstb.1994.0017

Stankowich, T., and Campbell, L. A. (2016). Living in the danger zone: exposure to predators and the evolution of spines and body armor in mammals. *Evolution (N. Y.)* 70, 1501–1511. doi: 10.1111/evo.12961

Stankowich, T., Caro, T., and Cox, M. (2011). Bold coloration and the evolution of aposematism in terrestrial carnivores. *Evol. Int. J. Org. Evol.* 65, 3090–3099. doi: 10.1111/j.1558-5646.2011.01334.x

Stephenson, P. J., and Racey, P. A. (1994). Seasonal variation in resting metabolic rate and body temperature of streaked tenrecs, *Hemicentetes nigriceps* and *H. semispinosus* (Insectivora: Tenrecidae). *J. Zool.* 232, 285–294. doi: 10.1111/j.1469-7998.1994.tb01573.x

Steven, A., and Seliger, B. (2018). The role of immune escape and immune cell infiltration in breast cancer. *Breast Care* 13, 16–21. doi: 10.1159/000486585

Sugiura, S. (2020). Predators as drivers of insect defenses. *Entomol. Sci.* 23, 316–337. doi: 10.1111/ens.12423

Sugiura, S., and Yamazaki, K. (2014). Caterpillar hair as a physical barrier against invertebrate predators. *Behav. Ecol.* 25, 975–983. doi: 10.1093/beheco/aru080

Tan, S., Li, D., and Zhu, X. (2020). Cancer immunotherapy: Pros, cons and beyond. *Biomed. Pharmacother.* 124:109821. doi: 10.1016/j.biopha.2020.109821

Taylor, A. R. (2011). *Emiliania Huxleyi Coccolithophore (PLoS). PLoS Biol., CC BY 2.5*. Available online at: https://commons.wikimedia.org/wiki/File:Emiliania_huxleyi_coccolithophore_(PLoS).png (accessed March 13, 2021).

Teng, M. W. L., Swann, J. B., Koebel, C. M., Schreiber, R. D., and Smyth, M. J. (2008). Immune-mediated dormancy: an equilibrium with cancer. *J. Leukoc. Biol.* 84, 988–993. doi: 10.1189/jlb.1107774

Thomson, D. A. (1964). Ostracitoxin: an ichthyotoxic stress secretion of the boxfish, *Ostracion lentiginosus*. *Science* 146, 244–245. doi: 10.1126/science.146.3641.244

Thurlow, L. R., Hanke, M. L., Fritz, T., Angle, A., Aldrich, A., Williams, S. H., et al. (2011). *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. *J. Immunol.* 186, 6585–6596. doi: 10.4049/jimmunol.1002794

van Donk, E., and Hessen, D. O. (1993). Grazing resistance in nutrient-stressed phytoplankton. *Oecologia* 93, 508–511. doi: 10.1007/bf00328958

Vareki, S. M. (2018). High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. *J. Immunother. Cancer* 6:157.

Villemagne, M. (2008). *Photo 56639549. iNaturalist, CC BY-NC 4.0*. Available online at: https://www.inaturalist.org/photos/56639549 (accessed March 13, 2021).

Vinay, D. S., Ryan, E. P., Pawelec, G., Talib, W. H., Stagg, J., Elkord, E., et al. (2015). Immune evasion in cancer: mechanistic basis and therapeutic strategies. *Semin. Cancer Biol.* 35, S185–S198.

Vito, A., El-Sayes, N., and Mossman, K. (2020). Hypoxia-driven immune escape in the tumor microenvironment. *Cells* 9:992. doi: 10.3390/cells9040992

Wagner, J., Wickman, E., DeRenzo, C., and Gottschalk, S. (2020). CAR T cell therapy for solid tumors: bright future or dark reality? *Mol. Ther.* 28, 2320–2339. doi: 10.1016/j.ymthe.2020.09.015

Waldbauer, G. (2012). *How Not to Be Eaten: The Insects Fight Back*. Berkeley, CA: University of California Press.

Walker, R., and Enderling, H. (2016). From concept to clinic: mathematically informed immunotherapy. *Curr. Probl. Cancer* 40, 68–83. doi: 10.1016/j.currproblcancer.2015.10.004

Wallace, A. R. (1867). Mimicry, and other protective resemblances among animals (1867). *Westminster Rev.* 32, 1–43.

Wallace, A. R. (1895). *Natural Selection and Tropical Nature: Essays on Descriptive and Theoretical Biology*. London: Macmillan and Company.

Wang, J., Xu, Y., Huang, Z., and Lu, X. (2018). T cell exhaustion in cancer: mechanisms and clinical implications. *J. Cell. Biochem.* 119, 4279–4286. doi: 10.1002/jcb.26645

Wang, L.-Y., Huang, W.-S., Tang, H.-C., Huang, L.-C., and Lin, C.-P. (2018a). Too hard to swallow: a secret secondary defence of an aposematic insect. *J. Exp. Biol.* 221:jeb172486.

Wang, L.-Y., Rajabi, H., Ghoroubi, N., Lin, C.-P., and Gorb, S. N. (2018b). Biomechanical strategies underlying the robust body armour of an aposematic weevil. *Front. Physiol.* 9:1410. doi: 10.3389/fphys.2018.01410

Weaver, A. M. (2006). Invadopodia: specialized cell structures for cancer invasion. *Clin. Exp. Metastasis* 23, 97–105. doi: 10.1007/s10585-006-9014-1

Weinstein, S. B. (2020). *Displaying*. Available online at: https://smithsonian.figshare.com/articles/media/Displaying/12185397?backTo=/collections/Lophio

mys_imhausi_crested_rat_behavior_in_captive_and_natural_environments/4948773 (accessed March 14, 2021).

Weinstein, S. B., Malanga, K. N., Agwanda, B., Maldonado, J. E., and Dearing, M. D. (2020). The secret social lives of African crested rats, *Lophiomys imhausi*. *J. Mammal.* 101, 1680–1691. doi: 10.1093/jmammal/gyaa127

Wetzel, B., and Schaefer, H. (1980). *Breast Cancer Cell, AV-8000-0302-B. National Cancer Institute*. Available online at: https://visualsonline.cancer.gov/details.cfm?imageid=1989 (accessed March 13, 2021).

Whelan, C. J., Holmes, R. T., and Smith, H. R. (1989). Bird predation on gypsy moth (Lepidoptera: Lymantriidae) larvae: an aviary study. *Environ. Entomol.* 18, 43–45. doi: 10.1093/ee/18.1.43

Wildes, T. J., Dyson, K. A., Francis, C., Wummer, B., Yang, C., Yegorov, O., et al. (2020). Immune escape after adoptive T-cell therapy for malignant gliomas. *Clin. Cancer Res.* 26, 5689–5700. doi: 10.1158/1078-0432.ccr-20-1065

Wishingrad, V., Musgrove, A. B., Chivers, D. P., and Ferrari, M. C. O. (2015). Risk in a changing world: environmental cues drive anti-predator behaviour in lake sturgeon (*Acipenser fulvescens*) in the absence of predators. *Behaviour* 152, 635–652. doi: 10.1163/1568539x-00003246

Witz, B. W., and Mushinsky, H. R. (1989). Pygidial secretions of *Pasimachus subsulcatus* (Coleoptera: Carabidae) deter predation by *Eumeces inexpectatus* (Squamata: Scincidae). *J. Chem. Ecol.* 15, 1033–1044. doi: 10.1007/bf01015197

Wood, W. F., Sollers, B. G., Dragoo, G. A., and Dragoo, J. W. (2002). Volatile components in defensive spray of the hooded skunk, *Mephitis macroura*. *J. Chem. Ecol.* 28, 1865–1870.

Wursten, B. (2017). *Photo 80983346. iNaturalist*. Available online at: https://www.inaturalist.org/photos/80983346 (accessed March 13, 2021).

Xu, J., Hansen, P. J., Nielsen, L. T., Krock, B., Tillmann, U., and Kiørboe, T. (2017). Distinctly different behavioral responses of a copepod, *Temora longicornis*, to different strains of toxic dinoflagellates, *Alexandrium* spp. *Harmful Algae* 62, 1–9. doi: 10.1016/j.hal.2016.11.020

Yang, W., Gludovatz, B., Zimmermann, E. A., Bale, H. A., Ritchie, R. O., and Meyers, M. A. (2013). Structure and fracture resistance of alligator gar (*Atractosteus spatula*) armored fish scales. *Acta Biomater.* 9, 5876–5889. doi: 10.1016/j.actbio.2012.12.026

Yang, W., Naleway, S. E., Porter, M. M., Meyers, M. A., and McKittrick, J. (2015). The armored carapace of the boxfish. *Acta Biomater.* 23, 1–10. doi: 10.1016/j.actbio.2015.05.024

Zarour, H. M. (2016). Reversing T-cell dysfunction and exhaustion in cancer. *Clin. Cancer Res.* 22, 1856–1864. doi: 10.1158/1078-0432.ccr-15-1849

Zerpe, R. (2018). *Thornback cowfish (Lactoria fornasini)*. *Wikimedia Commons*. Available online at: https://commons.wikimedia.org/wiki/File:Thornback_cowfish_(Lactoria_fornasini)_(43629194360).jpg (accessed March 13, 2021).

Zhu, D., Ortega, C. F., Motamedi, R., Szewciw, L., Vernerey, F., and Barthelat, F. (2012). Structure and mechanical performance of a "modern" fish scale. *Adv. Eng. Mater.* 14, B185–B194.

Zhu, J., Petit, P.-F., and Van den Eynde, B. J. (2019). Apoptosis of tumor-infiltrating T lymphocytes: a new immune checkpoint mechanism. *Cancer Immunol. Immunother.* 68, 835–847. doi: 10.1007/s00262-018-2269-y

Zintzen, V., Roberts, C. D., Anderson, M. J., Stewart, A. L., Struthers, C. D., and Harvey, E. S. (2011). Hagfish predatory behaviour and slime defence mechanism. *Sci. Rep.* 1:131.

Zvereva, E. L., and Kozlov, M. V. (2016). The costs and effectiveness of chemical defenses in herbivorous insects: a meta-analysis. *Ecol. Monogr.* 86, 107–124.

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frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 06 August 2021
doi: 10.3389/fevo.2021.681121

# Evolutionary Dynamics of Treatment-Induced Resistance in Cancer Informs Understanding of Rapid Evolution in Natural Systems

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OPEN ACCESS

**Edited by:**
Frederick R. Adler,
The University of Utah, United States

**Reviewed by:**
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University of St Andrews,
United Kingdom
Chiara Villa,
University of St Andrews,
United Kingdom, in collaboration with reviewer MC
Christopher Carroll,
Lund University, Sweden

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**Specialty section:**
This article was submitted to Models in Ecology and Evolution, a section of the journal Frontiers in Ecology and Evolution

**Received:** 15 March 2021
**Accepted:** 23 June 2021
**Published:** 06 August 2021

**Citation:**
Pressley M, Salvioli M, Lewis DB, Richards CL, Brown JS and Staňková K (2021) Evolutionary Dynamics of Treatment-Induced Resistance in Cancer Informs Understanding of Rapid Evolution in Natural Systems. Front. Ecol. Evol. 9:681121. doi: 10.3389/fevo.2021.681121

Rapid evolution is ubiquitous in nature. We briefly review some of this quite broadly, particularly in the context of response to anthropogenic disturbances. Nowhere is this more evident, replicated and accessible to study than in cancer. Curiously cancer has been late - relative to fisheries, antibiotic resistance, pest management and evolution in human dominated landscapes - in recognizing the need for evolutionarily informed management strategies. The speed of evolution matters. Here, we employ game-theoretic modeling to compare time to progression with continuous maximum tolerable dose to that of adaptive therapy where treatment is discontinued when the population of cancer cells gets below half of its initial size and re-administered when the cancer cells recover, forming cycles with and without treatment. We show that the success of adaptive therapy relative to continuous maximum tolerable dose therapy is much higher if the population of cancer cells is defined by two cell types (sensitive vs. resistant in a polymorphic population). Additionally, the relative increase in time to progression increases with the speed of evolution. These results hold with and without a cost of resistance in cancer cells. On the other hand, treatment-induced resistance can be modeled as a quantitative trait in a monomorphic population of cancer cells. In that case, when evolution is rapid, there is no advantage to adaptive therapy. Initial responses to therapy are blunted by the cancer cells evolving too quickly. Our study emphasizes how cancer provides a unique system for studying rapid evolutionary changes within tumor ecosystems in response to human interventions; and allows us to contrast and compare this system to other human managed or dominated systems in nature.

**Keywords: metastatic cancer, adaptive therapy, evolutionary speed, resistance, game theory, Stackelberg evolutionary game**

## 1. INTRODUCTION

Organisms can respond rapidly to contingencies and changes in their environment. When they cannot extinction may follow. The Stephens Island wren was flightless and free of mammalian predators until the lighthouse keeper introduced Tibbles the house cat (Galbreath and Brown, 2004; Medway, 2004). Extinction of the wren followed shortly thereafter. The birds either did not or could not muster behavioral responses, and did not have the time needed to evolve appropriate responses. Similarly, the introduction of brown tree snakes (*Boiga irregularis*) has threatened a number of birds, bats and reptiles on islands such as Guam leading to dramatic losses of species diversity (Savidge, 1987; Fritts and Rodda, 1998; Wiles et al., 2003). On the other hand, many species respond quickly to dramatic changes in their environment or even colonize novel environments. Examples include responses to size selective harvesting of fish (Conover and Munch, 2002; Salvioli et al., 2021); re-emergence of anti-predator behaviors with the reintroduction of predators (Laundré et al., 2001); rapid evolution of body size, behavior and other traits in invasive organisms (Huey et al., 2000; Whitney and Gabler, 2008; Turner et al., 2014; Vandepitte et al., 2014; Rollins et al., 2015; Selechnik et al., 2019) or members of invaded communities (Chapuis et al., 2017); and shifts in reproduction and migration in animals and plants in response to climate (Parmesan et al., 1999; Franks et al., 2007; Geerts et al., 2015).

Rapid evolution may permit species to adjust to rapid changes in their environments, but rapid evolution can also be consequential to human welfare and health. For example, herbicide and pesticide resistance threatens the productivity of crops (Kuester et al., 2014; Baucom, 2019; Hawkins et al., 2019). The boll weevil (*Anthonomus grandis* Boheman) was reported as a serious pest in U.S. cotton production as far back as 1892 and developed resistance to insecticides within a few years in the 1950's (Perkins, 1980). Today, boll weevil control involves an integrated pest management (IPM) approach using pheromone traps and insecticides timed around the weevil's reproductive cycles (Shipman, 2017). For upwards of 50 years now, IPM strategies have employed resistance management plans which can include application of targeted pheromones and allelochemicals, leading to a reduction in the use of broad-spectrum insecticides and ensuring that non-target and beneficial insects are not adversely affected (Tewari et al., 2014; Brown and Staňková, 2017; Cunningham, 2019). Similarly, drug resistance poses direct threats to patient health. Chloroquine resistant strains of malaria have become particularly prevalent in West Africa and Papua New Guinea (Wellems and Plowe, 2001). Antibiotic resistance threatens the advances that have been made in controlling infectious diseases. The emergence of methicillin-resistant Staphylococcus aureus (MRSA) strains of pathogenic bacteria have been the subject of epidemiological, experimental and mathematical-modeling studies (Robinson and Enright, 2003).

While the idea that cancer progression is an evolutionary process has been discussed for several decades (Cairns, 1975; Nowell, 1976), the application of ecological and evolutionary principles to understanding rapid evolution in cancer has only recently become a major objective [e.g., the classic by Nowell (1976) has been cited over 7,000 times in Google Scholar; more than half of that in the last 10 years]. Cancers provide a unique study of rapid evolution because within a matter of months or years, cancer within its host will evolve adaptations for evading the immune system, increasing vasculature, co-opting the signaling pathways of normal cells, and gathering scarce nutrients more quickly and efficiently (Hanahan and Weinberg, 2000, 2011). This trajectory of extremely rapid evolution begins *de novo* in each patient. In addition to the rapid evolution of cancer in the host, resistance to therapy can also evolve quickly (Dujon et al., 2020; Gatenby and Brown, 2020a). When metastatic, like the cotton boll weevil, the cancer will evolve resistance to all available drugs. Cancer then represents a microcosm for studying and managing rapid evolution that is replicated across patients (Pienta et al., 2020).

There are some important differences between studying rapid evolution in ecosystems compared to in human disease, and cancer in particular. Evolutionary speed in wild populations, for instance, can be sensitive to sex ratios that determine effective population sizes (Allendorf et al., 2008), an issue that does not apply to asexual reproduction through mitotic cell division. For antibiotic resistance, the fear is not that the current patient will succumb because of rapid evolution, but that the application of antibiotics across millions of patients will result in a strain emerging from a subset of these patients that will go on to infect others (Ventola, 2015). In contrast, the concern in cancer is not that a resistant strain will jump from patient to patient. Instead, the problem lies entirely within the patient and the eco-evolutionary dynamics that lead to therapy failure.

Here, we are interested in addressing and modeling the consequences of two features of eco-evolutionary models of adaptive therapy (AT). These features are the speed of evolution, and whether the cancer cell population is monomorphic (where treatment-induced resistance evolves as a quantitative trait) vs. polymorphic (sensitive vs. resistant cell types where treatment-induced resistance evolves only in resistant cells, also as a quantitative trait). In what follows, we elaborate more fully on the determinants of evolutionary speed (section 2) and the broader contexts of rapid evolution (section 3). In section 4, we introduce therapeutic strategies in cancer as a special form of integrated pest management. We then develop a model of therapy that includes the ecological dynamics of tumor burden and the evolutionary dynamics of changes in the composition of cancer cell types within the patient. We analyze the consequences of evolutionary speed in determining the efficacy of a standard form of AT relative to continuous drug delivery at maximum tolerable dose (MTD) (section 5). We model this in the context of monomorphic and polymorphic cancer cell populations, and in the context of having no cost of resistance, a cost of resistance manifested in intrinsic growth rates, and a cost of resistance manifested in the carrying capacities. Section 6 concludes by summarizing the main outcomes of the cancer model and discussing how our results could be transferable to other fields. In addition to adding to the modeling results for AT in cancers, we hope to show evolutionary biologists and ecologists just how

similar resistance management in cancer is to managing evolving species (that may be pests or resources), and to show cancer biologists how the challenge of therapy resistance is kindred to conservators and managers of biodiversity and pests in nature.

## 2. DETERMINANTS OF EVOLUTIONARY SPEED

A better understanding of the evolution of resistance to therapy in cancer can be informed by theory and examples of rapid evolution in several ecological contexts. Evolution occurs over many timescales. The domain of evolutionary science classically has involved taxa with vertical inheritance, so discussions of "rapid" evolution by many scholars emphasize the surprisingly small number of generations over which substantial changes in heritable phenotypes are observed. Hairston et al. (2005) defined rapid evolution as "genetic change occurring rapidly enough to have a measurable impact on simultaneous ecological change." While the authors emphasize the change must be genetic, their analysis is actually based on heritable phenotypic change which could result from genetic, epigenetic or other forms of non-genetic inheritance (Bonduriansky and Day, 2009; Jablonka and Raz, 2009; Keller, 2014; Müller, 2017; Stoltzfus, 2017; Banta and Richards, 2018; Richards and Pigliucci, 2020; Mounger et al., 2021). Evolution is known to occur rapidly in wild populations abruptly subjected to novel selection pressures. Rapid evolution is well-documented in invasive populations (Bock et al., 2015; van Kleunen et al., 2018; Mounger et al., 2021), and wild populations experiencing intensive human intervention related to urbanization, agro-ecosystem management, wild species harvest, and pollution (Sullivan et al., 2017).

Researchers have long been interested in the mechanisms that allow for these rapid responses to environmental challenges. The frequencies of heritable phenotypes in wild populations may change within only a few generations when novel environmental conditions are highly lethal to some portion of existing trait variation. Sudden ecological and climatic changes are particularly effective at driving rapid phenotypic change and underlying change in genetic and non-genetic inheritance mechanisms. In response to climate change in the UK, for instance, some populations of the brown argus butterfly (*Aricia agestis*) have shifted female preference for host plant species, and exhibited reduced fitness in ancestral habitats within 10–15 years of the shift (Buckley and Bridle, 2014). Plenty of evidence suggests that these responses can be in part dictated by classic expectations of selection acting on genetic diversity (Hoffmann and Sgro, 2011). The type of intense selection that induces rapid evolutionary change, however, may be accompanied by a loss in genetic diversity and heritable variation.

On the other hand, many invasive species offer important counter evidence to the assumption that reduced genetic variance indicates reduced evolutionary potential (Colautti and Lau, 2015; Dlugosch et al., 2015; Stapley et al., 2015; Estoup et al., 2016; Selechnik et al., 2019). The population bottlenecks inherent to invasion have long been assumed to hinder evolutionary potential creating the "genetic paradox" of invasion (Estoup et al., 2016; Mounger et al., 2021), but recent studies have shown that in fact the genetic paradox may not be as severe as initially thought. This is due to a myriad of genomic possibilities. First, many invasive populations undergo only modest reductions in genetic variation due to multiple introductions, hybridization or *de novo* mutations (Estoup et al., 2016). But importantly, loss of genetic diversity measured by molecular markers does not reflect loss of quantitative trait variation or may reflect selection of fit genotypes or recombination among founding genotypes (e.g., Selechnik et al., 2019). Genetic bottlenecks can also contribute to performance by purging deleterious alleles, revealing beneficial cryptic variation or creating new beneficial interactions among genomic elements (Colautti and Lau, 2015; Dlugosch et al., 2015; Stapley et al., 2015; Estoup et al., 2016; van Kleunen et al., 2018).

In addition to genetic variants, the plasticity of morphological, physiological and behavioral traits are clearly important (West-Eberhard, 1989; Richards et al., 2006; Lankau, 2011; Ledón-Rettig et al., 2013; Rollins et al., 2015). Theoretical work suggests putative upper limits on rates of genetic evolution, and that rapid trait changes result in part from phenotypic plasticity (Kopp and Matuszewski, 2014). The distinction is complex, since phenotypic plasticity is genetically based but also underlain by epigenetic mechanisms that can be independent of genetic differences (Richards et al., 2006, 2010, 2017; Cortijo et al., 2014; Banta and Richards, 2018). Furthermore, the molecular-level mechanisms that contribute to such plastic responses can ultimately lead to genetic changes or non-genetic inheritance (West-Eberhard, 1989; Bonduriansky and Day, 2009, 2018; Klironomos et al., 2013; Kronholm and Collins, 2016; Kronholm et al., 2017; Wölfl et al., 2020). A particularly striking example of the disconnect between genetic variation and heritable phenotypic response is in the single octoploid clone of Japanese knotweed that has spread aggressively through a broad range of habitats in temperate Europe and North America (Beerling et al., 1994; Bailey and Conolly, 2000; Grimsby et al., 2007; Gerber et al., 2008; Bailey et al., 2009; Richards et al., 2012; Zhang et al., 2016). Several studies have linked the divergence in these populations to differences in DNA methylation (Richards et al., 2008, 2012; Zhang et al., 2016). Despite the potential importance of this type of clonal spread particularly in invasive plant species, our ability to understand the roles for existing mutations, *de novo* mutations, and epigenetics remains constrained by too few studies (Paun et al., 2019; Richards and Pigliucci, 2020; Mounger et al., 2021).

Extensive genomics studies in cancer have revealed that "genetic instability" is a hallmark of cancer (Coffey, 1998; Duesberg et al., 1998). While no universal driver mutations of metastases have been identified, Gerstung et al. (2020) demonstrated by analysis of 2,658 samples of 38 different cancers types in the Pan-Cancer Analysis of Whole Genomes (PCAWG) that very early events in cancer are limited to a common set of drivers. In fact, 50% of early mutations in cancers occur in just 9 genes. Mutations in epigenetic machinery can also be important in shaping genome dynamics in cancer (Feinberg et al., 2006; Timp and Feinberg, 2013). In particular, chromatin regulators are often mutated in cancer. Mutations in the SWI/SNF complex occur in over 20% of all cancers (Kadoch and Crabtree, 2015).

Recent studies indicate that specific genetic mutations can instigate metastases but that completion of the process depends only on non-genetic changes, specifically epigenetic changes that complement the genetic mutations (Lambert et al., 2017).

The molecular basis of trait variation can have important impacts on the speed of evolution as evidenced by studies in herbicide and pesticide resistance. Hawkins et al. (2019) recently compared three major pesticide groups (insecticides, herbicides, and fungicides) to make this point. They argued that fungicide resistance evolves more often by *de novo* point mutations in functional genes, herbicide resistance evolves through selection on standing variation; and insecticide resistance evolves through a combination of standing variation and *de novo* mutations. The rate at which resistance evolves in these groups depends on the dynamics within the populations. They argue that *de novo* mutation must spread through the movement of insects, seeds, pollen, or spores, whereas a preexisting allele may already be present throughout the range. These arguments suggest that the pathway to resistance can determine the most effective containment strategy (Hawkins et al., 2019).

## 3. CONTEXTS OF RAPID EVOLUTION

Humans are selective agents that influence the evolution of non-human wild populations by harvesting them, attempting to suppress or extirpate them, and by altering their biophysical environment (Hendry et al., 2017). These anthropogenic interventions often induce trait changes more rapidly and to a greater extent than observed in the evolution of populations inhabiting more natural contexts (Hendry et al., 2008), although even natural populations can undergo rapid evolution in real time (Weiner, 1995; Reznick et al., 2019). Human interventions drive evolution in traits such as body size in harvested fish (Olsen et al., 2004; Salvioli et al., 2021), and dispersal traits of urban plants (Cheptou et al., 2008). Accelerated rates of evolution may result from shifts in the adaptive landscapes resulting from anthropogenic changes or from increased variance in relative fitness among individuals (Fugère and Hendry, 2018). Human-induced evolutionary change may render populations resistant to future management and may endow populations with functional traits that feed back to affect the properties of ecosystems that benefit people (Rudman et al., 2017). This rapid evolution of management-resistant traits, and feedbacks on the health of ecosystems, are analogous to the management of therapy resistance, tumor burden, and patient well-being in oncological settings. Like the clinician and cancer, the practitioner/manager (e.g., in an agroecosystem or fishery) and non-human population (e.g., of weeds or fish) coevolve, one through rational decision making and one through selection (Staňková et al., 2019; Salvioli et al., 2021). Next, we briefly highlight some principles developed through the study of rapid evolution in anthropogenic contexts of urbanization, weed management in agrosystems, and wild animal harvest.

### 3.1. Urbanization

Urban evolutionary ecology and adaptive cancer therapies share a common interest in populations responding to large magnitude environmental changes. Urbanization is characterized by a host of changes in the biophysical environment, including accelerated cycling of nutrients and pollutants, altered energy budgets that induce warming via heat island effects, landscape fragmentation and the proliferation of impervious surface, modified soil structure and fertility, redistributions of water, changes to physical architecture, and homogenization of ecological communities and the attendant introduction of novel competitor, predator, and pathogen species (Grimm et al., 2008). Populations of plants, animals, and microorganisms experience this wide portfolio of changes under non-equilibrium conditions, as urbanization generates perpetual changes in environments rather than stable endpoints (Collins et al., 2000). Cancer cell populations, likewise, experience dramatic environmental change either when therapies are imposed on extant tumors, or during metastasis as cells migrate to distinct areas of the body. Like the environmental changes that constitute urbanization, adaptive therapies seek to impose non-equilibrium selection regimes on cancer populations to disrupt the emergence, or dominance of resistant cancer cells.

Urban populations can exhibit sufficient trait variation for rapid evolution. For instance, variation in plant size and allocation traits among urban plant species often exceeds that of their non-urban conspecifics (Borowy and Swan, 2020). Urban environmental conditions alter phenotype frequencies in non-human wild populations by inducing plastic responses, as well as through both adaptive and non-adaptive evolutionary changes (Johnson and Munshi-South, 2017). For instance, acorn ants (*Temnothorax curvispinosus*) reared from urban populations inhabiting environments warmed 2°C by the heat island effect show higher heat tolerance and narrower thermal tolerance breadths than ants reared from rural populations. Yet, even rural ants can develop higher heat tolerance through acclimation, indicating both fixed and plastic phenotypic responses to the urban thermal environment (Diamond et al., 2017).

Landscape fragmentation in cities can isolate small populations, reducing gene flow and promoting genetic drift, resulting in potentially non-adaptive genetic differentiation among populations. Transcriptome differences among distinct urban populations of white-footed mice (*Peromyscus leucopus*) occupying isolated habitat patches in New York, for instance, suggest that both selection and genetic drift account for rapid evolutionary responses to urbanization (Harris et al., 2013). Populations inhabiting urban environments can exhibit adaptive changes in sexually selected traits when compared with their conspecific rural counterparts (Yeh, 2004). Important questions remain about the extent to which urban environmental properties induce mutation or affect genome-wide mutation rates, and whether adaptation to urban environments results more often from mutations that occur after populations are urbanized or from standing, pre-urban genetic variation (Barrett and Schluter, 2008). These pressing questions for non-human populations adapting to urbanization are similarly relevant for cancer cell populations evolving responses to diverse tumor microenvironments and therapy-induced selection.

Urban evolutionary ecology investigates not only phenotypic responses of populations to urban environmental conditions,

but also examines how these altered populations and their traits affect urban ecosystem processes (Alberti, 2016). Ecosystems carry out processes such as primary production of biomass and organic energy, decomposition and nutrient recycling, hosting of biodiversity, and societally valued services like storm energy mitigation, food production, pollutant capture, and recreation. The degree and manner in which ecosystems carry out these processes depend strongly on the functional traits of an ecosystem's constituent species (Rudman et al., 2017). These traits are the products of evolution occurring under the selective regime imposed by ecosystem processes, setting up reciprocal eco-evolutionary feedbacks between population traits and the environment in which those traits emerged. Similarly, through mechanisms such as promoting vascularization, acidic pH, and cancer associated fibroblasts, cancer cells evolve traits that alter their environment creating eco-evolutionary feedbacks (De Groot et al., 2017).

## 3.2. Agroecosystem Weed Management

Tumor cells and agricultural pests both form undesirable populations that humans attempt to eradicate or manage through the application of biocides. Parallels to cancer therapies are arguably most evident in the battle against weed plants and other pests in agroecosystems. Pesticides and antibiotics, among other agricultural technologies, have afforded increases in food supply necessary for a growing and urbanizing human population. But, the intensive application of these chemicals select for resistance in weeds, insect pests, and crop and livestock pathogens (Pittendrigh et al., 2013; Kuester et al., 2014; Baucom, 2019). One strategy developed in cropping systems to inhibit rapid evolution of resistance in weeds is the application of herbicide mixtures (Wrubel and Gressel, 1994). This mixture approach is distinct from the sequential use of multiple herbicides, one at a time, until each has selected for resistance in the focal weed population. It is also distinct from the application of multiple herbicides that each target a separate weed species. Instead, this mixture approach consists of simultaneously applying multiple herbicides with different modes of action to control a single weed population. Similar approaches using multi-drug cocktails have become commonplace in cancer treatments. Toxicity to patients often dictates the doses and combinations of drugs that can be safely administered.

Theory and experience reveal several criteria for delaying the evolution of herbicide resistance in weeds (Wrubel and Gressel, 1994), with parallels comparable to mixed-therapy strategies in oncology that likewise seek to delay the rapid evolution of resistance in tumor cell populations. First, both (or more) herbicides in a mixture must control the same weed population, as an herbicide having no effect on a focal weed population will not influence the rate at which it evolves resistance to another herbicide. Second, both herbicides must be similarly effective in killing weeds (e.g., lethal to similar percentages of the focal weed population), and third, both must persist in the environment for similar durations; failure to meet these criteria leaves some portion of the focal weed population exposed to only one of the herbicides and thus prone to rapid evolution of resistance to it. Fourth, the two herbicides must have different biochemical targets within the focal weed population, such as inactivating different proteins or enzyme systems. Fifth, both must be degraded through different mechanisms; failure to meet these criteria may induce evolution of cross-resistance. Although the use of herbicide mixtures effectively inhibits the evolution of specialist resistance traits, one downside of this strategy appears to be selection for generalist resistance (Comont et al., 2020). Whether this outcome is unavoidable or results from failure to meet the above criteria is unclear, but it does raise a warning sign for extrapolating biocide mixture approaches to oncology. Lastly, a desirable property of mixtures is negative cross-resistance, in which one herbicide selects for alleles that confer hypersusceptibility to the other herbicide. In cancer, such negative cross-resistance are known as double-bind therapies in which drugs should be given sequentially rather than together (Gatenby et al., 2009a; Basanta et al., 2012; Gatenby and Brown, 2020b).

Pollutants, although not intentionally applied to wild populations, can mimic pesticides by acting as lethal poisons. As such, like pesticides, they have the capacity to impose intense selective pressure and drive rapid evolution. In temperate and boreal regions, for example, salts used to de-ice roads commonly run off into freshwater ecosystems. The resulting salinization of these water bodies raises the question of whether freshwater populations can adapt to the selective pressure imposed by this new water chemistry regime. Indeed, populations of the freshwater cladoceran zooplankton *Daphnia*, a critical link in most freshwater lake food webs, can adapt to higher (albeit not extreme) salinities in 5–10 generations (Coldsnow et al., 2017).

## 3.3. Harvested Animal Populations

Humans harvest wild animal populations to obtain food, furs and clothing materials, ornamental features such as horns and antlers, and collectable specimens (e.g., mollusc shells). Given these motivations, animals with particular traits or trait values (e.g., particular morph or size) are often targeted for harvesting, driving phenotypic change in harvested populations. Phenotypic responses to harvesting are well-documented in fishes, from freshwater recreational harvesting (Sutter et al., 2012) to marine commercial harvesting (Law, 2000), and in a wide variety of ungulates such as bighorn sheep (Pigeon et al., 2016) and elephants (Jachmann et al., 1995). More pervasively, selective harvest and associated phenotypic change is also documented in a variety of other mammalian and invertebrate taxa (Allendorf et al., 2008). Harvest reduces the frequency of desirable phenotypes in populations, quite opposite to the reinforcement of desired phenotypes under artificial selection in agriculture and aquaculture (Allendorf and Hard, 2009). While lack of additive genetic variance and plasticity in targeted traits may limit heritable responses to harvest, harvest is generally thought to drive evolution through three mechanisms: reduced local densities that open harvested subpopulations to immigration and concurrent genetic swamping and loss of local adaptation, selection on standing variants, and reduced genetic variation (Allendorf et al., 2008). Molecular genetic monitoring is recommended to detect harmful genetic change that results from selective mortality via harvest (Allendorf and Hard, 2009).

Assessing and monitoring for specific mutations or overall genetic heterogeneity have become part of personalized medicine in cancer treatments. Genetic predispositions of the patient and the presence of specific driver mutations often permit early detection of cancer, indicate the presence of certain types of drug resistance, and dictate the course of therapy.

While there is consensus that harvesting changes phenotypic frequencies in harvested populations, the role of evolution in these changes remains uncertain, particularly in harvested fish populations. Harvest may indeed act on heritable variation and thereby drive evolution, but may also induce ecological changes (e.g., reduced population densities) that provoke plastic responses in harvested populations (Kuparinen and Festa-Bianchet, 2017). Some evidence suggests that adaptation is localized and occurs rapidly (within a few generations). Age-structured population models indicate that harvest strategies that ignore harvest-induced evolution can ultimately depress sustained yields because they irreversibly select for maturation at smaller sizes and younger ages (Heino, 1998). Probabilistic maturation reaction norms delineate the probability (usually 50%) of maturation for different combinations of age and size. Changes in the shape of reaction norms (as opposed to changes in location along a reaction norm) indicate evolutionary change, and, when such shifts coincide with harvest, provide evidence of harvest-induced evolution (Olsen et al., 2004). Converse arguments posit that relatively low heritability of relevant life history traits means that evolutionary responses to fish harvesting may require long time scales (Law, 2007). Evolutionary responses to harvesting may be difficult to detect because of counter-gradient variation, where, for example, alleles for fast growth are favored in cold environments, and vice versa, thereby reducing phenotypic variation among populations across environmental (e.g., climatic) gradients (Jorgensen et al., 2007).

A principle concern for wildlife managers is whether evolutionary responses to selective mortality via harvest undermines the ecological sustainability of the harvested population. Fishing, for example, may select for traits that are not adaptive with respect to natural and sexual selection regimes, leaving harvested populations without the phenotypic traits or variation needed to cope with their environment (Conover, 2000). Adaptive variation needed to recover during fishing moratoria may be limited in overharvested fish populations. For instance, overharvested populations of many species exhibit reduced allelic diversity and heterozygosity (Pinsky and Palumbi, 2014). Moreover, harvesting diminishes traits that correspond with fitness (weapon size in ungulates, size at maturity, and boldness in fish), yet when harvest is suspended to permit recovery, countervailing selection that favors the reemergence of these traits may be less intense, prolonging population recovery (Allendorf et al., 2008). This outcome was evident in northern populations of Atlantic cod (*Gadus morhua*), in which harvest selected for a younger age and smaller size at maturity. Population densities remained depressed even after a decade of fishing moratorium Olsen et al. (2004). While diminished sustainability of a wild population is an undesirable outcome of evolutionary responses to selective mortality, analogous outcomes in oncological settings would be favorable. Useful therapies may be those that impose evolutionary trade-offs on cancer cell populations by selecting for cancer cell phenotypes that are maladaptive to the natural selection regime imposed by the immune system, or that intensely select against antagonistic traits that are only modestly favored during AT holidays. These are key issues in designing, implementing and modeling AT.

## 4. MODELING ECO-EVOLUTIONARY DYNAMICS OF CANCER IN RESPONSE TO TREATMENT

Similar to human intervention in ecology, therapeutic intervention in cancer can favorably or unfavorably direct evolution. MTD is the standard of care in which therapy is given continuously for a predetermined amount of time. When MTD is unable to eliminate all cancerous cells it inevitably selects for the continued proliferation of treatment-resistant cells (Chabner and Roberts, 2005; Gatenby, 2009; Pepper et al., 2009; Aktipis et al., 2011; Greaves and Maley, 2012). In contrast, AT modulates therapy based on tumor dynamics in response to treatment. When there is a cost to resistance (a disadvantage to being resistant), therapy-sensitive cells outcompete their resistant counterparts in the absence of treatment. Therefore, drug withdrawal during AT suppresses the ability for resistant cells to dominate the tumor population (Gatenby et al., 2009b; Zhang et al., 2017; Staňková et al., 2019). The evolutionary capacity of cancer cell phenotypes to withstand therapy induced selection regulates the effectiveness of therapy. Specifically, how fast or slow evolution occurs may play a key role in therapeutic success. We develop mathematical models to analyze the impact of evolutionary speed on the success of AT when compared to MTD.

Combating resistance is prevalent in nature and medicine when dealing with an evolving population. Resistance mechanisms to biocides in pest species and therapies in cancer can be categorized as follows: (1) strictly qualitative (for instance, the presence of an upregulated or novel metabolic pathway in the resistant form), which can be modeled using a polymorphic population where one strain is sensitive while another strain possesses a resistance trait which is fixed and does not evolve; (2) strictly quantitative (for instance, the production of binding or detoxification enzymes, and for cancer and microorganisms an increase in the number of membrane pumps for eliminating the toxin), which can be modeled using a monomorphic population with an evolving resistance trait; or (3) a hybrid combination of contexts 1 and 2, which can be modeled using a polymorphic population where one strain remains sensitive while another strain possesses an evolving quantitative resistance trait. For example, in the case of abiraterone resistance in metastatic castrate-resistant prostate cancer there are three qualitative cell types: those requiring exogenous testosterone, those independent of testosterone, and those producing testosterone as a public good (You et al., 2017, 2019; Zhang et al., 2017). While the first two are strictly qualitative, the last type can also be quantitative in terms of the amount of testosterone produced. Strictly qualitative resistance traits have been explored in detail by modelers (You

et al., 2017; Zhang et al., 2017; West et al., 2018; Cunningham et al., 2020; Bayer et al., 2021; Kim et al., 2021; Viossat and Noble, 2021), strictly quantitative less so (Staňková et al., 2019; Reed et al., 2020; Salvioli, 2020; Wölfl et al., 2020), and the combination, to our knowledge, has not been explored at all. Our models can be used to consider all three contexts (strictly qualitative, strictly quantitative or both). In what follows, we shall focus on comparing contexts 2 with 3. We do not model context 1 (polymorphic population, strictly qualitative resistance) as it is a special case of our context 3 (polymorphic population with resistance evolving as a quantitative trait of the resistant population). A final key element for all considerations of evolving pests and managed species concerns a cost of resistance. A cost of resistance will slow the evolution of resistance, and render more sensitive types more competitive than less sensitive types when the biocide is removed. In our models, we consider what happens when there is no cost of resistance, a cost of resistance in the intrinsic growth rate, or a cost of resistance in the carrying capacity.

Our models are generic in the sense that they can frame biological systems other than cancer and their eco-evolutionary responses to management by humans. However, we narrow our model analysis to cancer. We consider the superiority of AT relative to MTD as measured by time to progression (TTP). We consider jointly the effects of evolutionary speed, the context of resistance, and the nature of the cost of resistance. The model permits any level and spacing of dosing, but we focus on contrasting a continuous, fixed level of dosing with a form of AT where the dosing is either on or off depending on the patient's tumor burden.

## 4.1. Our Models

We model the evolution of resistance leading to treatment failure using ordinary differential equations (ODEs) for a monomorphic (context 2) and polymorphic (context 3) tumor cell population. In our monomorphic model, resistance is a quantitative trait. The resistance strategy exists on a continuum, and all cells can exhibit some magnitude of resistance $u(t)$, which evolves in time. In our polymorphic model, we assume the entire tumor cell population is comprised of two distinct subpopulations, sensitive and resistant cells. In this model, only the resistant cell subpopulation has the capacity to evolve resistance as a quantitative trait $u_R(t)$. **Table 1** displays all scenarios for each model and **Table 2** indicates all parameters and their definitions. In the following, we assume that the tumor populations grow logistically and are suppressed by the presence of therapy and natural cell turnover.

All models describe Darwinian dynamics of cancer in response to treatment, with a fitness-generating function, "G-function" (Vincent and Brown, 2005). A G-function considers how the fitness of a focal cancer cell using a strategy $v$ in the population is influenced by the environment and by the strategies and population sizes of the resident phenotypes. The set of phenotypic strategies present in the tumor are represented by $\mathbf{u}$. The population size of cells with a particular strategy is indicated by $\mathbf{x}$. In the polymorphic context, the vector $\mathbf{u} = (u_R, u_S)^T$ encompasses the strategy for resistant and sensitive cells and $\mathbf{x} = (x_R, x_S)^T$ their population sizes. In the monomorphic context $\mathbf{u}$ and $\mathbf{x}$ are reduced to scalars as only a single evolutionary strategy defines the entire tumor population. We assume that the physician applies a treatment dose $m(t) \in [0, 1]$ at time $t \geq 0$, where $m(t) = 0$ and $m(t) = 1$ correspond to no dose and MTD at time $t$, respectively. For simplicity, the drug is assumed to be maximally effective at MTD. The efficacy of the drug is reduced by a focal cell's resistance strategy $v$, innate drug immunity $k$, and the benefit $b$ of the resistance trait in reducing therapy efficacy. The G-function is used to derive the evolutionary dynamics that describe how the resident strategies (i.e., phenotypes) of the tumor change with time. Note that in this case the fitness function for a rare mutant does not directly depend on the current resident strategies. Following Fisher's fundamental theorem of natural selection, the resistance strategies change in the direction of the fitness gradient $\frac{\partial G}{\partial v}$ with respect to the fitness of a rare mutant $v$ (Fisher, 1930). This derivative is then evaluated at the current resident strategies $\mathbf{u}$, giving an equation defining the evolutionary dynamics for each resident strategy (**Table 1**) (Vincent and Brown, 2005). The rate at which the strategies change is scaled by an evolutionary speed term $\sigma$. In our model, large values of evolutionary speed $\sigma$ correspond to enhanced phenotypic variance which could result from increased genetic variance or phenotypic plasticity. Innate immunity $k$ suggests that prior to drug exposure cells possess a mechanism that inhibits the potency of treatment. This parameter is the only value that reduces drug efficacy for the sensitive population in our polymorphic model as the sensitive cells cannot evolve resistance. Treatment efficacy is further diminished by the magnitude of the benefit $b$ of the resistance strategy for the monomorphic population and the resistant population in the polymorphic model. For a general introduction to our modeling framework, see **Appendix B**.

Although resistance decreases treatment efficacy, it may be that a resistance strategy comes at a cost (Staňková, 2019). When a cost to resistance is present, resistance confers a selective advantage during treatment. In the absence of therapy, a cost of resistance confers a fitness disadvantage. In our model, we consider that resistance either carries no cost ($K(v) = K_{\max}$, $r(v) = r_{\max}$), carries a cost in the intrinsic growth rate ($r(v) = r_{\max}\, e^{-g\, v}$, $K(v) = K_{\max}$), or carries a cost in the carrying capacity ($r(v) = r_{\max}$, $K(v) = K_{\max}\, e^{-g\, v}$). These costs are relevant when modeling the monomorphic population, and for the resistant population when modeling the polymorphic context.

## 4.2. Case Studies

We analyze the impact of two treatment strategies (MTD and AT) on TTP. We define TTP as the first time at which the tumor burden reaches $\delta = 70\%$ of the maximum carrying capacity $K_{\max}$, during treatment.

The treatment schedule for each strategy is as follows:

- Maximum tolerable dose (MTD): $m(t) = 1$ for all $t$;
- Adaptive therapy (AT): Initially, MTD is administered ($m(0) = 1$) until the tumor cell population size $x$ reaches half of its initial density. Treatment is then discontinued until the tumor recovers to its initial size where treatment is re-administered beginning a new treatment cycle (Zhang et al.,

**TABLE 1 |** Different models analyzed in this paper: The first, second, and third lines of this table describe eco-evolutionary cancer dynamics with no cost of resistance, cost of resistance in the growth rate, and cost of resistance in the carrying capacity of cancer cells, respectively.

| Resistance | Monomorphic cancer population | Polymorphic cancer population |
|---|---|---|
| None | $G(v,u,x,m) = r_{\max}\left(1-\frac{x}{K_{\max}}\right) - d - \frac{m}{k+bv}$<br>$\dot{x} = x\,G(v,u,x,m)\big\vert_{v=u}$<br>$\dot{u} = \sigma\,\frac{\partial G(v,u,x,m)}{\partial v}\Big\vert_{v=u}$ | $G(v,\mathbf{u},\mathbf{x},m) = r_{\max}\left(1-\frac{\sum_{i\in\{R,S\}} x_i}{K_{\max}}\right) - d - \frac{m}{k+bv}$<br>$\dot{x}_i = x_i G(v,\mathbf{u},\mathbf{x},m)\big\vert_{v=u_i}$ where $i \in \{R,S\}$<br>$\dot{u}_R = \sigma\,\frac{\partial G(v,\mathbf{u},\mathbf{x},m)}{\partial v}\Big\vert_{v=u_R}$<br>$u_S = 0$ |
| $r(v) = r_{\max}e^{-gv}$ | $G(v,u,x,m) = r(v)\left(1-\frac{x}{K_{\max}}\right) - d - \frac{m}{k+bv}$<br>$\dot{x} = x\,G(v,u,x,m)\big\vert_{v=u}$<br>$\dot{u} = \sigma\,\frac{\partial G(v,u,x,m)}{\partial v}\Big\vert_{v=u}$ | $G(v,\mathbf{u},\mathbf{x},m) = r(v)\left(1-\frac{\sum_{i\in\{R,S\}} x_i}{K_{\max}}\right) - d - \frac{m}{k+bv}$<br>$\dot{x}_i = x_i G(v,\mathbf{u},\mathbf{x},m)\big\vert_{v=u_i}$ where $i \in \{R,S\}$<br>$\dot{u}_R = \sigma\,\frac{\partial G(v,\mathbf{u},\mathbf{x},m)}{\partial v}\Big\vert_{v=u_R}$<br>$u_S = 0$ |
| $K(v) = K_{\max}e^{-gv}$ | $G(v,u,x,m) = r_{\max}\left(1-\frac{x}{K(v)}\right) - d - \frac{m}{k+bv}$<br>$\dot{x} = x\,G(v,u,x,m)\big\vert_{v=u}$<br>$\dot{u} = \sigma\,\frac{\partial G(v,u,x,m)}{\partial v}\Big\vert_{v=u}$ | $G(v,\mathbf{u},\mathbf{x},m) = r_{\max}\left(1-\frac{\sum_{i\in\{R,S\}} x_i}{K(v)}\right) - d - \frac{m}{k+bv}$<br>$\dot{x}_i = x_i G(v,\mathbf{u},\mathbf{x},m)\big\vert_{v=u_i}$ where $i \in \{R,S\}$<br>$\dot{u}_R = \sigma\,\frac{\partial G(v,\mathbf{u},\mathbf{x},m)}{\partial v}\Big\vert_{v=u_R}$<br>$u_S = 0$ |

**TABLE 2 |** Variables and parameters of the model.

| | Meaning | Values |
|---|---|---|
| **Variables** | | |
| $x$ | Cancer cell population (monomorphic case) | In interval $[0, K_{\max}]$ |
| $x_S$ | Sensitive population (polymorphic case) | In interval $[0, K_{\max}]$ |
| $x_R$ | Resistant population (polymorphic case) | In interval $[0, K_{\max}]$ |
| $u$ | Resistance strategy (monomorphic case) | Non-negative |
| $v$ | Resistance strategy (focal individual) | Non-negative |
| $u_S$ | Sensitive population resistance strategy (polymorphic case) | 0 |
| $u_R$ | Resistant population resistance strategy (polymorphic case) | Non-negative |
| $m$ | Treatment dose | In interval [0, 1] |
| **Parameters** | | |
| $r_{\max}$ | Intrinsic growth rate of the cancer cells | 0.45 |
| $K_{\max}$ | Carrying capacity | 10,000 |
| $k$ | Innate cell immunity | 2 |
| $b$ | Magnitude of resistance benefit | 10 |
| $\sigma$ | Evolutionary speed | In interval (0, 1] |
| $\delta$ | Progression threshold (fraction of $K$) | 70% |
| $g$ | Magnitude of cost of resistance | In interval (0, 1] |
| $d$ | Intrinsic death rate | 0.01 |

2017). We will also, at times, consider a 20% reduction in tumor volume as the switch threshold. While this has not been tried in any clinical trial, we include it as several authors have shown that it gives superior results as compared to a 50% reduction (Kim et al., 2021; Strobl et al., 2021; Viossat and Noble, 2021).

We first consider the case of a monomorphic tumor cell population, which has evolved resistance $u(t)$ at time $t \geq 0$ in response to treatment $m(t)$. Here, $u(t) = 0$ corresponds to no resistance. We do not impose an upper bound on the resistance trait, thus $u(t)$ is a quantitative trait achieving non-negative values. For the sake of simplicity of expressions, we will drop the time variable $t$ whenever this does not compromise the clarity. For this case, we always consider initial conditions $x(0) = 6{,}000$ and $u(0) = 0$.

Next, we consider the case of a polymorphic tumor cell population. We assume that the tumor is composed of distinct sensitive $x_S$ and resistant $x_R$ subpopulations of cells. The sensitive subpopulation utilizes a fixed resistance strategy $u_S = 0$, and

this value does not evolve with time. The resistant subpopulation, initially expresses almost no resistance $u_R(0) = 0.01$ and evolves treatment-induced resistance ($u_R > 0$) over time. The initial conditions for each subpopulation are $x_S(0) = 5,990$, and $x_R(0) = 10$.

We also analyze how different assumptions regarding the cost of resistance in cancer cells (no cost of resistance, cost of resistance in cancer cells' growth rate ($r(v) = r_{\max} e^{-g v}$), and cost of resistance in cancer cells' carrying capacity ($K(v) = K_{\max} e^{-g v}$)) impact the success of MTD and AT in terms of TTP. Altogether, our modeling efforts investigate how the TTP varies between AT and MTD therapy, dependent on the following:

- The type of cancer population (monomorphic vs. polymorphic),
- The cost of resistance (none, on $r$, on $K$), and
- Evolutionary speed $\sigma$.

The models were solved numerically through the `odeint` function of the Python 3.6 Scipy package, using three-stage Adams-Bashforth method with adaptive stepsize and backward differentiation formula for stiff and non-stiff problems, respectively. All results were also duplicated in Mathematica, to validate their correctness.

## 5. RESULTS

In this section, we begin by investigating how the cost of resistance impacts tumor burden and TTP during continuous therapy at MTD. Subsequently, we compare TTP under MTD vs. AT for each of our eco-evolutionary models introduced in the previous section. The ability to extend TTP signifies a greater treatment efficacy and the treatment strategy exhibiting a longer TTP is deemed superior. We show that in all of our model scenarios, AT is superior to MTD. The magnitude of this superiority (increase in TTP compared to MTD) is influenced by the context (monomorphic vs. polymorphic tumor composition), evolutionary speed, and to a lesser extent by the cost of resistance.

### 5.1. A Cost of Resistance Manifested in the Carrying Capacity Comprises Tumor Growth the Most

Typically, MTD leads to an initial decrease in the tumor burden, followed by the evolution of resistance and ultimately treatment failure. **Figure 1** depicts these dynamics for both our polymorphic (top row) and monomorphic (bottom row) tumor cell populations exhibiting different resistance costs. For all cases, MTD results in evolutionary dynamics where there is a monotonic increase in the level of resistance of the evolving cancer cell populations. When there is a cost of resistance (influenced by parameter $g$), TTP increases, resistance evolves more slowly, and maximum tumor burden declines. In both population contexts, when there is no cost to resistance the tumor burden stabilizes at a population density 4% greater than when the cost of resistance is expressed in the intrinsic growth rate $r$ and almost 20% greater than when the cost of resistance is expressed in the carrying capacity $K$. Thus, a cost applied to the carrying capacity compromises tumor regrowth the most, leading to an extended TTP. As our intuition would suggest, when the cost of resistance is very high, the population dynamics stabilize at much lower tumor burdens (**Figure A1**). Based on model formulation and parameterization, there are instances when tumor regrowth stabilizes at a survivable tumor burden that does not result in progression. In **Figure 1**, the tumor burdens of both population contexts recover to reach what we consider disease progression, however in the monomorphic context, the initial decrease in the population density is much less than in the polymorphic context. In some cases, continuous therapy will not reduce the tumor burden sufficiently to allow for AT. For the purposes of our investigation, we focus on scenarios where continuous treatment results in at least a 50% decline in the initial tumor burden, and where resistance evolution under continuous therapy will result in a tumor burden that exceeds $\delta$=70% of the maximum carrying capacity (which we consider to represent disease progression). With these two stipulations, we restrict ourselves to conditions where AT can be applied, and where continuous MTD will eventually result in disease progression.

### 5.2. AT Improves TTP With or Without a Cost of Resistance, Where the Improvement Is Greatest When the Cost Is Applied to the Carrying Capacity

Independent of the presence of a cost of resistance, AT is superior to MTD when we do not consider evolutionary speeds. In **Figure 2**, we see that in the polymorphic context AT leads to a clear improvement in treatment efficacy in terms of TTP with respect to MTD. The left panel exhibits the population dynamics for this polymorphic population during each treatment schedule and the right panel displays the strategy dynamics of the resistant cell type. TTP is represented by red stars which identify when the tumor burden reaches 7,000. Regardless of the resistance cost, AT extends TTP compared to MTD. In the absence of a resistance cost, AT increases TTP by 33%. With a cost of resistance, AT increases TTP by 34 and 45% when manifested in the growth rate and carrying capacity, respectively. Drug holidays during AT lead to disruptions in the strategy dynamics of the resistant cells, increasing the time it takes for them to reach levels of resistance that result in disease progression. We further analyze how therapy influences the frequency of sensitive and resistant cells within the tumor in **Figure 3**. Here we observe the dynamics of each subpopulation and the total population of cancer cells with MTD (top row) and AT (bottom row). There is no decrease in the resistant subpopulation when therapy is not applied, but a drug holiday through adaptive scheduling decreases the speed of its growth. When therapy is applied continuously there is no opportunity for the sensitive subpopulation to regrow and maintain a tumor composition that is majority drug sensitive. We note that eventually, also with AT, the resistant subpopulation outcompetes the sensitive one and the disease progresses. Furthermore, we explore a parameterization that allows us to compare AT to MTD for a monomorphic and polymorphic population (**Figure A2**). The dynamics are similar to **Figure 2**, AT remains advantageous for

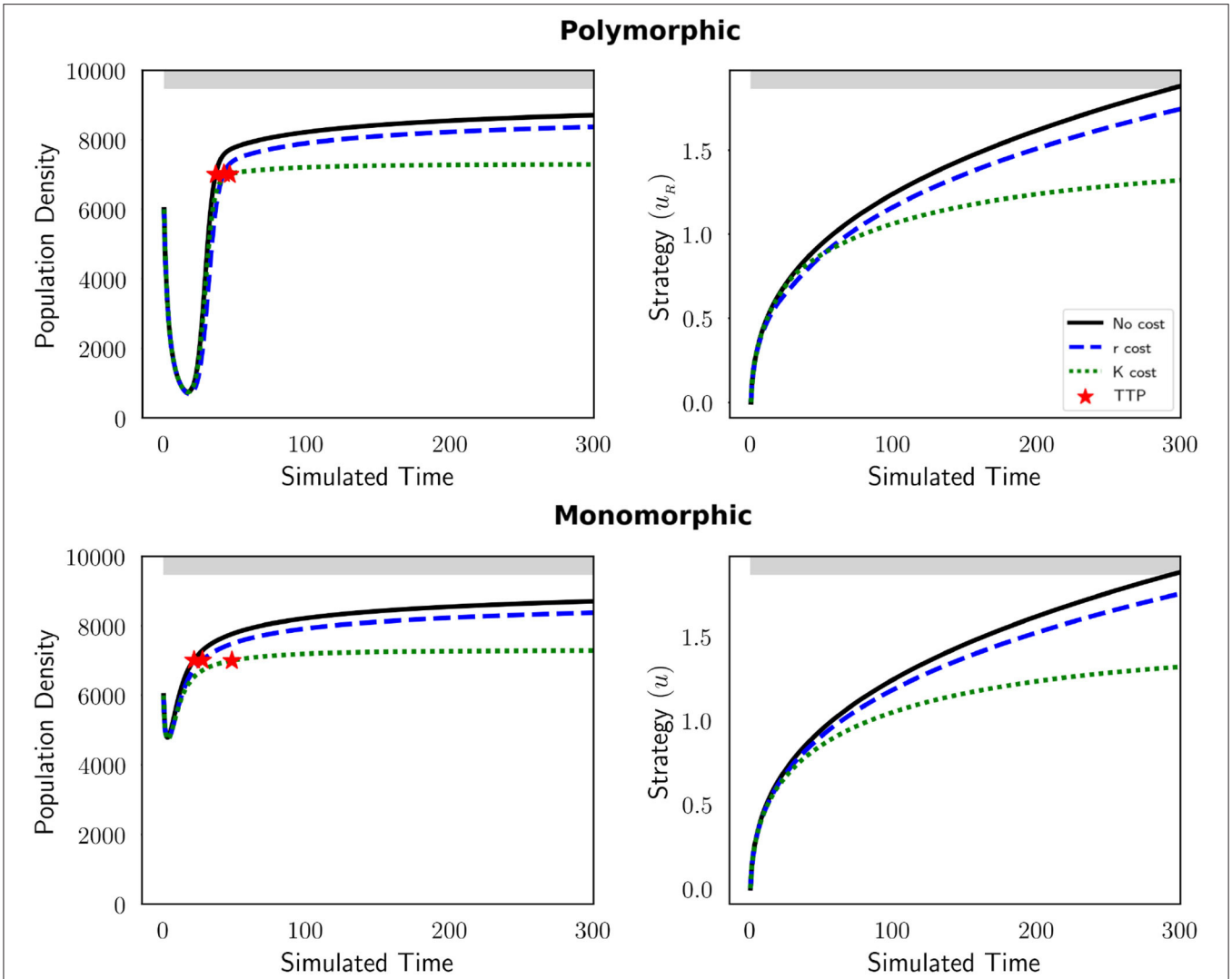


**FIGURE 1 |** The presence of a resistance cost can improve the short-lived therapeutic success of MTD treatment. Drug administration is shown at the top of each plot in gray. Population dynamics **(left)** and the evolutionary dynamics **(right)** are shown in the absence of a resistance cost (black solid line), and when the cost is manifested in the intrinsic growth rate (blue dashed) and carrying capacity (green dot dashed line) for a polymorphic **(top)** and monomorphic **(bottom)** tumor cell population. The strategy in the polymorphic context is that of the resistant population of cells. Red stars indicate progression defined as $x \geq$7,000. When a cost of resistance is imposed on carrying capacity there is a greater extension in TTP. The parameters used in these figures are: $m = 1$, $K_{\max} = 10,000$, $r_{\max} = 0.45$, $k = 2$, $b = 10$, $d = 0.01$, $g = 0.1$, $\sigma = 0.1$.

both contexts. The TTP for all cases is increased significantly due to a reduction in the cost of resistance ($g = 0.01$) and benefit of resistance ($b = 1$).

## 5.3. Faster Speeds of Evolution Reduce the Improvement in TTP Provided by AT

Evolutionary speed $\sigma$ contributes to the effectiveness of AT. At faster speeds of evolution, tumor regrowth occurs quicker, decreasing TTP (**Figure A3**). The impact of increasing evolutionary speed on TTP for MTD and AT in a polymorphic population is shown in **Figure 4**. At very slow speeds of evolution, we see significantly longer TTPs for all contexts of resistance, both for MTD and AT. We observe again that when the resistance cost is manifested in carrying capacity of resistant cells, TTP is longer, for both MTD and AT, than when resistance cost is manifested in intrinsic population growth rate or when there is no cost. When $\sigma > 0.01$ the relative TTP for each treatment strategy decreases dramatically. Nonetheless, under all evolutionary speeds and resistance cost scenarios, AT remains superior to MTD via lengthening TTP (**Figure 4**).

In absolute terms ($TTP_{AT} - TTP_{MTD}$) this benefit declines as evolutionary speed increases, while proportionally ($(TTP_{AT} - TTP_{MTD})/TTP_{MTD}$), the benefit of AT increases with evolutionary speed (**Figure 5**).

Relative to MTD, AT will extend TTP by a greater proportion at faster evolutionary speeds due to the already short-lived

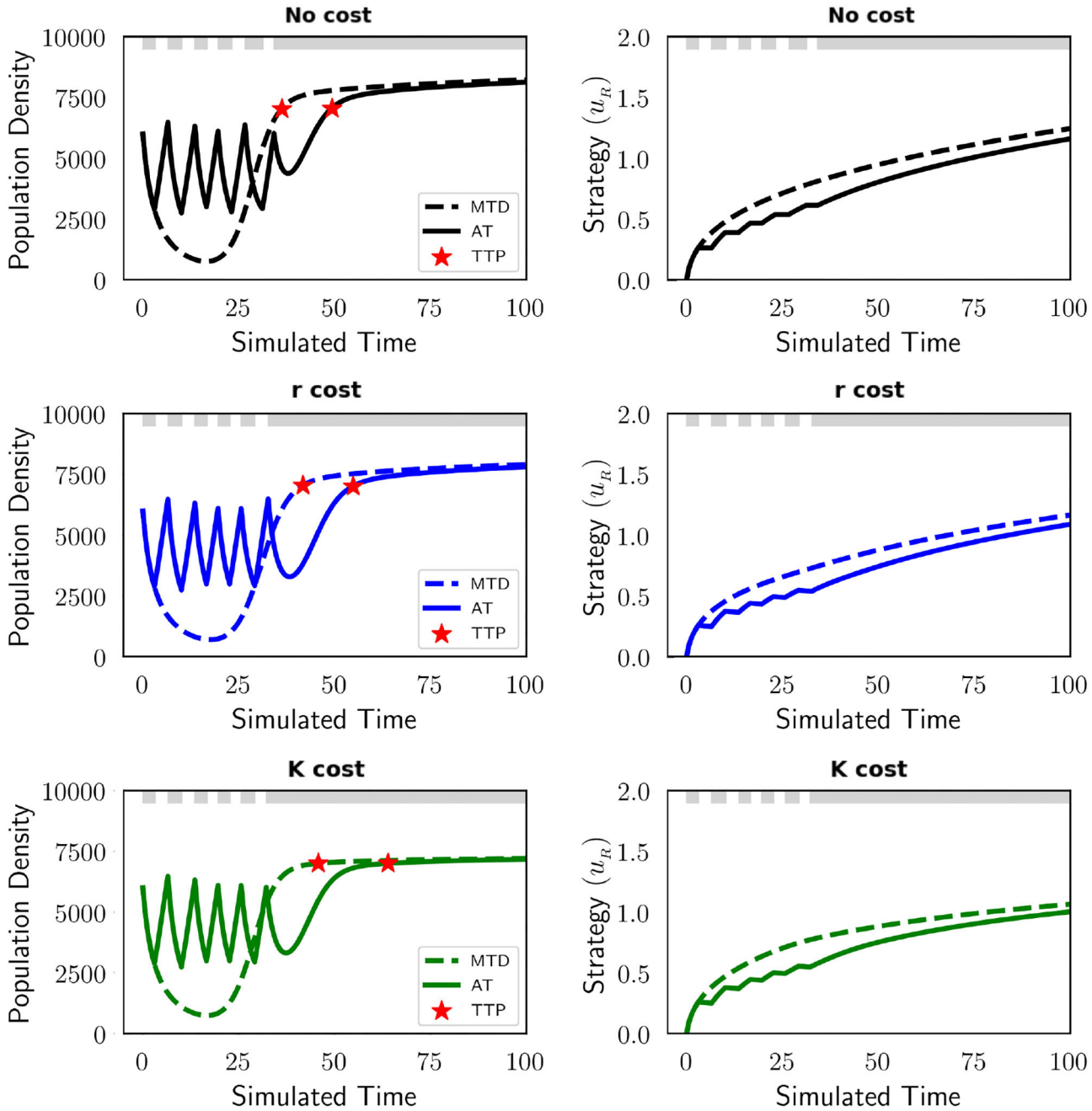


**FIGURE 2 |** AT is superior to MTD independent of the cost of resistance. Simulations of AT (solid line) and MTD (dashed line) for a polymorphic population are shown in the absence of a resistance cost (black), and when the cost impacts the intrinsic growth rate (blue) and carrying capacity (green). Population dynamics **(left)** and evolutionary dynamics **(right)** illustrate the advantage of AT compared to MTD. The protocol for AT is to withdraw the drug once the population density $\leq 3,000$ (50% of the initial population size). Drug administration for AT is shown at the top of each plot in gray. TTP for each is illustrated by red stars. Only the strategy for the resistant population is shown as the sensitive population has a fixed strategy ($u_s = 0$). Regardless of the cost of resistance, AT always corresponds to a longer TTP. The parameters used in this figure are: $m = 1, K_{\max} = 10,000, r_{\max} = 0.45, k = 2, b = 10, d = 0.01, g = 0.1, \sigma = 0.1$.

therapeutic success of MTD at those greater speeds. AT does not confer a proportionally large increase in TTP when TTP under MTD is relatively long. Under this parameterization, AT relative to MTD provides a greater proportional improvement in TTP when a resistance cost is expressed in carrying capacity than when it is expressed in population growth rate or not at all. However, when evolutionary speed is fast, this advantage is lost. Since the original AT trial protocol was introduced, subsequent studies suggest that withholding therapy sooner provides a greater benefit. We explore this by removing treatment once the tumor

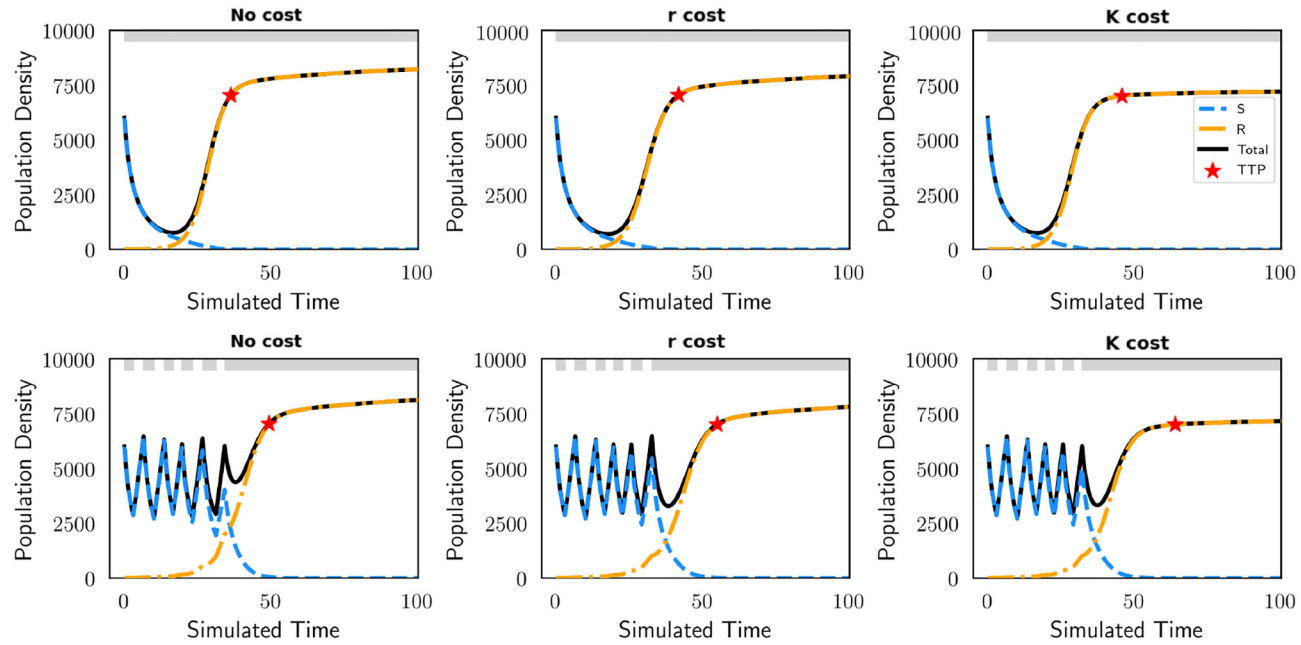


**FIGURE 3 |** Changes in the frequency of sensitive (blue) and resistant (yellow) subpopulations in the polymorphic context during MTD **(top)** and AT **(bottom)**. At the top of each plot, drug administration is shown in gray. These dynamics are shown for three different models of costs of resistance: no cost, cost applied to the growth rate (*r* cost), and cost applied to the carrying capacity (*K* cost). AT delays TTP (red star) by maintaining a tumor composition of mostly sensitive cells for a longer time than MTD. The parameters used in this figure are: $m = 1, K_{max} = 10,000, r_{max} = 0.45, k = 2, b = 10, d = 0.01, g = 0.1, \sigma = 0.1$.

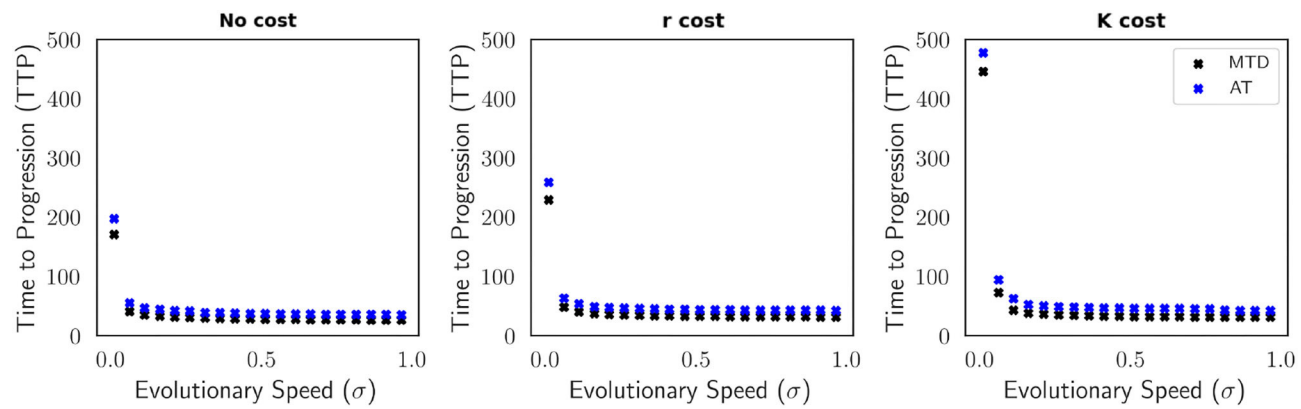


**FIGURE 4 |** TTP for MTD (black) and AT (blue) as a function of the evolutionary speed for a polymorphic tumor cell population. This is illustrated for each model of cost of resistance (no cost, *r* cost, and *K* cost). At faster speeds of evolution, AT remains favorable but TTP decreases for both AT and MTD. Of the three models of cost of resistance, the TTP for MTD and AT is shortest in the absence of a cost and greatest when the cost of resistance impacts the carrying capacity *K*. The parameters used in this figure are: $m = 1, K_{max} = 10,000, r_{max} = 0.45, k = 2, b = 10, d = 0.01, g = 0.1$.

burden drops 20% of initial density (**Figure 5B**). This change in protocol amplifies the results shown when using a 50% threshold. The overall trends remain the same, the relative superiority of AT is greater at faster speeds of evolution.

## 5.4. Improvement in TTP Provided by AT Is Greater for a Polymorphic Population

As previously stated, the AT regimen cannot be applied to a monomorphic population using the same parameterization. This is due to an insufficient decrease in tumor burden during continuous treatment (**Figure 1**). By reducing the benefit ($b$ is decreased from 10 to 1) and cost of resistance ($g$ is decreased from 0.1 to 0.01) we are able to achieve population dynamics during MTD that satisfy our requirements for AT. In **Figure 6**, we compare improvement in TTP when AT is applied to the polymorphic and monomorphic contexts, and to different forms of the cost of resistance. As before, having a cost of resistance in carrying capacity produces a longer TTP than having no cost

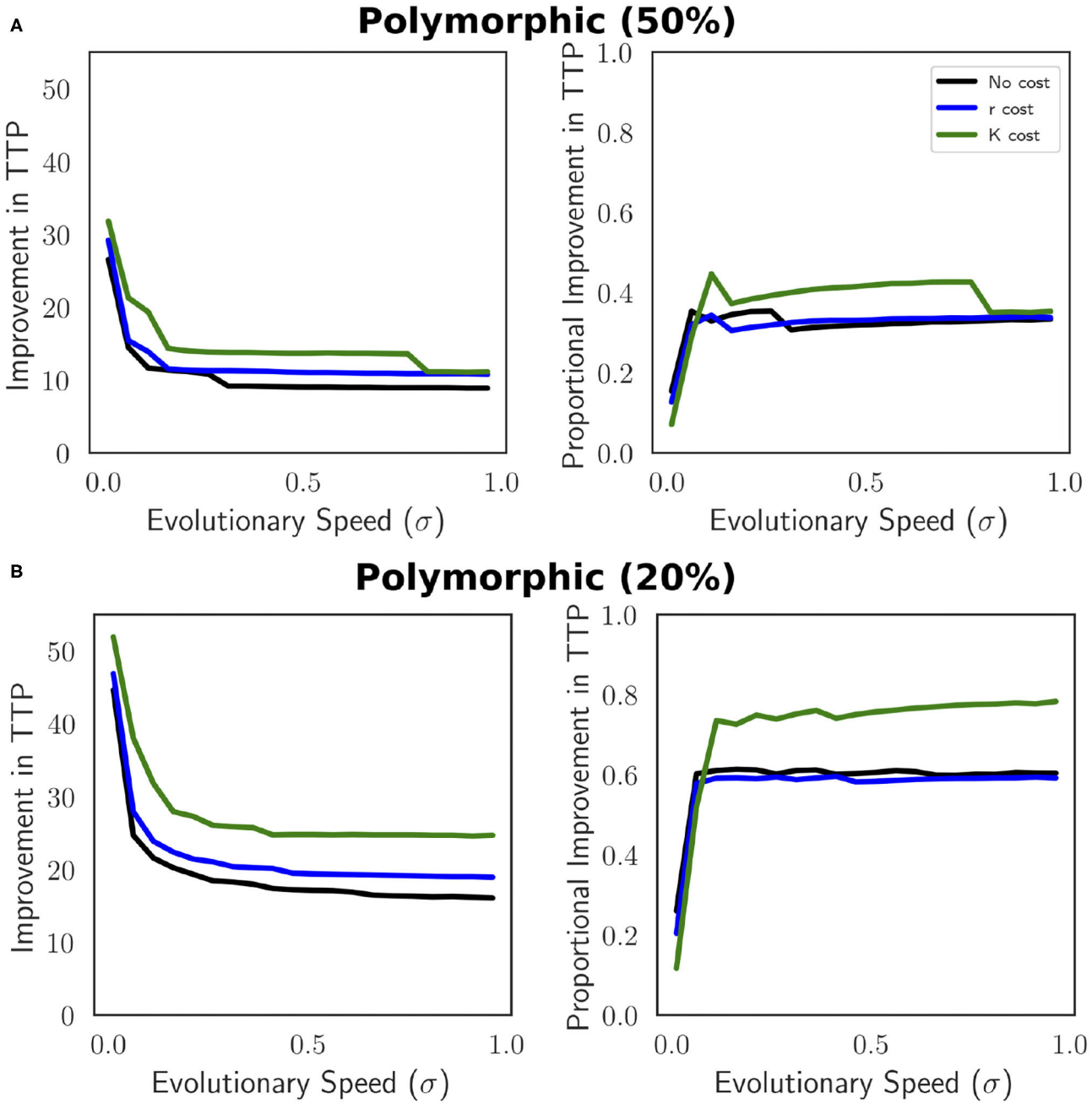


**FIGURE 5 |** Improvement in TTP ($TTP_{AT} - TTP_{MTD}$) and proportional improvement in TTP ($(TTP_{AT} - TTP_{MTD})/TTP_{MTD}$) as a function of evolutionary speed for a polymorphic tumor cell population. **(A)** Therapy is withdrawn once the population density $\leq 3,000$ (50% of the initial population size) and re-administered once the population $\geq 6,000$ (initial population size). **(B)** The threshold to remove therapy is reduced from 50% to 20%. Therapy is withdrawn once the population density $\leq 4,800$ (20% decrease from initial population size) and re-administered once the population $\geq 6,000$ (initial population size). The benefit of AT increases at faster speeds of evolution. A reduction in the magnitude the tumor population decreases before therapy can be withdrawn makes AT more effective. The parameters used in this figure are: $m = 1, K_{\max} = 10,000, r_{\max} = 0.45, k = 2, b = 10, d = 0.01, g = 0.1$.

or a cost in intrinsic population growth rate (**Figure A4**). Here we observe a trend similar to **Figure 5** in terms of improvement in TTP for both population contexts, independent of the cost of resistance. As evolutionary speed increases the improvement AT provides decreases.

Our results show that there is a significant difference in the improvement AT bestows based on tumor composition. Improvement in TTP with AT is not as advantageous for a monomorphic population as it is for a polymorphic population. The proportional improvement of AT is minimal

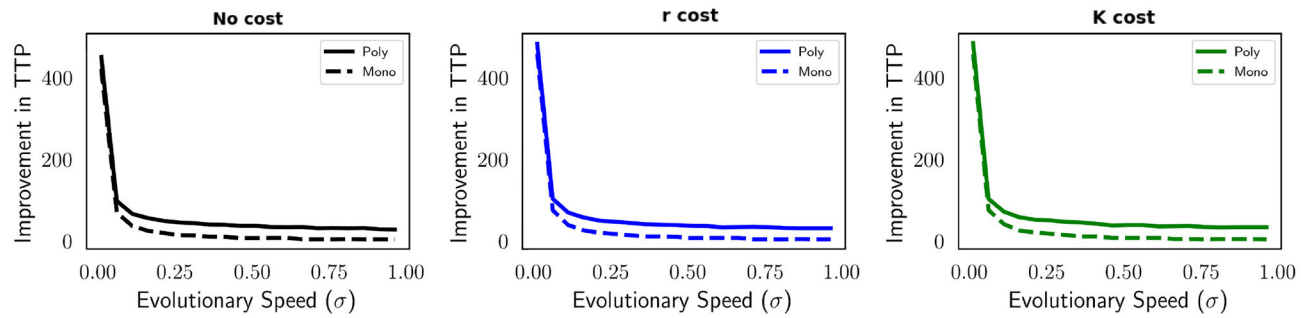


**FIGURE 6 |** The impact of evolutionary speed on the improvement in TTP for a monomorphic and polymorphic tumor cell population compared between different models of cost of resistance. Compared to **Figures 1–5**, $b$ is reduced from 10 to 1 and $g$ is reduced from 0.1 to 0.01. Rapid evolution decreases the improvement in TTP provided by AT independent of a resistance cost. Despite the decline, there remains a greater effectiveness of AT when the population is polymorphic. All parameter values used in this figure are: $m = 1, K_{\max} = 10,000, r_{\max} = 0.45, k = 2, b = 1, d = 0.01, g = 0.01$.

when the population is monomorphic (**Figure 7**). At faster speeds of evolution, the relative superiority of AT decreases in this population. In contrast, there is a greater proportional improvement in TTP under AT than under MTD when the population is polymorphic than when it is monomorphic. The proportional AT advantage is shown to correlate positively with evolutionary speed. In the polymorphic case, the proportional improvement in TTP under AT vs. under MTD is greatest where TTP under MTD is short (**Figure 5**). The altered therapy protocol (**Figure 7B**), again, shows that the overall results remain the same. Notably, maintaining the tumor burden at a therapy switching threshold of 20% its original size allows AT to be more effective. Although rapid evolution is unfavorable in terms of delaying TTP, AT is proportionally more successful when the evolutionary speed is faster.

# 6. DISCUSSION

## 6.1. Main Outcomes

In this paper, we model how the speed of evolution of treatment-induced resistance in cancer cells impacts the patient's TTP under two treatment regimens: (i) maximum tolerable dose (MTD) and (ii) adaptive therapy (AT) following the Zhang protocol, where MTD is discontinued when the tumor reaches half of its initial volume and is re-administered only once the tumor recovers (Zhang et al., 2017). We considered two eco-evolutionary contexts. In the first, there is a monomorphic population of cancer cells, with treatment-induced resistance being a quantitative trait that evolves in accord with standard models of quantitative genetics and adaptive dynamics. In the second, the population of cancer cells is assumed to be polymorphic with a strictly sensitive subpopulation that does not evolve, and a resistant subpopulation of cancer cells that can evolve increasing resistance as a quantitative trait.

There is an existing tradition of cancer models that treat the evolving trait of the cancer cells as quantitative in ordinary differential equation models (Orlando et al., 2012; Staňková et al., 2019; Reed et al., 2020; Salvioli, 2020), partial differential equation models (Lorenzi et al., 2016; Almeida et al., 2019) and in agent-based models (Gallaher et al., 2018). There is also a tradition of modeling resistance evolution by presupposing pre-existing populations of therapy resistant and sensitive populations (Sun et al., 2016; You et al., 2017; Zhang et al., 2017; Cunningham et al., 2020; Strobl et al., 2020; Kim et al., 2021; Viossat and Noble, 2021). In these models, resistance is a qualitative trait and it does not exist on a continuum. Our second model and eco-evolutionary context represents a new approach to the resistance strategy. Like models with a quantitative trait, we let the resistant population start with a positive but low level of the resistance trait. This subpopulation evolved its quantitative trait according to Darwinian dynamics. Like models with qualitative traits we assumed a sensitive, non-evolving subpopulation that had only some level of innate resistance to the therapy. It remains an open empirical question in all fields that involve managing evolving pests and resources whether the population under management is (1) polymorphic with sensitive and resistant subpopulations with fixed values for their resistance traits, (2) monomorphic with a quantitative trait, or (3) polymorphic with an evolving resistant population and a fixed sensitive population. We suspect examples of all three exist in nature, in disease management, and in pest management. For the second and third eco-evolutionary contexts (where in fact the first context mentioned is a special case of the third one), we considered three different modeling forms for the cost of resistance: (i) no cost of resistance, (ii) the cost of resistance manifests as a decrease in the growth rate of cancer cells $r$, and (iii) the cost of resistance manifests as a decline in the cancer cells' carrying capacity $K$.

Our first main observation concerns the eco-evolutionary context with a monomorphic cancer population with a continuous resistance trait. While AT outperforms MTD, it does so only very slightly and only at slow evolutionary speeds. Above a certain rate of evolution, the tumor burden never decreases to half of its initial volume and as such, AT TTP coincides with that of standard of care. This outcome is independent of whether or not the cost of resistance is considered. TTP is slower with a cost of resistance than without. In terms of the proportional increase of the TTP when compared to the MTD regimen, AT with no cost of resistance outperforms AT when the cost of resistance acts on $r$ (intrinsic growth rate) which outperforms AT when the

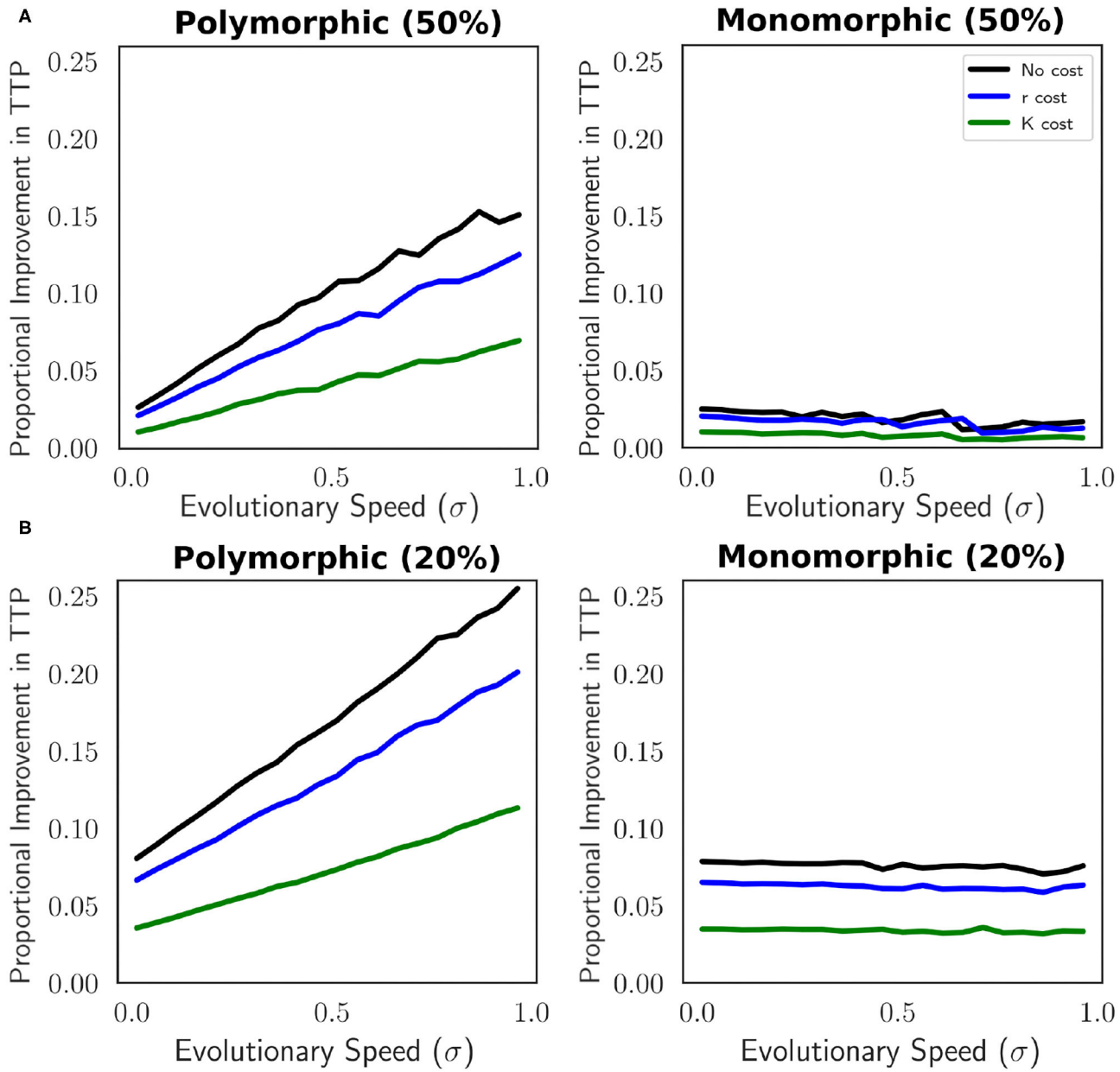


**FIGURE 7 |** Comparison of proportional improvement in TTP as a function of evolutionary speed for a monomorphic and polymorphic tumor cell population. Three models of resistance are compared: no cost of resistance, cost of resistance expressed in $r$, and cost of resistance expressed in $K$. **(A)** Therapy is withdrawn once the population density $\leq 3,000$ (50% of the initial population size) and re-administered once the population $\geq 6,000$ (initial population size). **(B)** The threshold to remove therapy is reduced thus from 50% to 20%. Therapy is withdrawn once the population density $\leq 4,800$ (20% decrease from initial population size) and re-administered once the population $\geq 6,000$ (initial population size). The superiority of AT when the population is monomorphic is minimal and as evolutionary speed increases the relative benefit of AT declines. A polymorphic population of cells allows for a greater effectiveness of AT. In this case, when the speed of evolution is quick AT provides a greater proportional benefit. Withdrawing therapy at 20% opposed to 50% improves the proportional increase in the effectiveness of AT. The parameters used in this figure are: $m = 1, K_{\max} = 10,000, r_{\max} = 0.45, k = 2, b = 10, d = 0.01, g = 0.1$.

cost of resistance impacts $K$ (carrying capacity). The relatively lackluster benefits of AT under a quantitative resistance strategy has to do with the fitness gradients of the adaptive landscape. The slope of the logistic term of the fitness function is either a drag on evolving resistance when therapy is on or the force that evolves greater sensitivity when therapy is off. This aspect of the fitness gradient is always active. Not so for the effect of therapy. The mortality term from therapy is active and decisive in evolving resistance when therapy is on, but it becomes neutral, not negative, when therapy is off. Thus, in general, therapy being on propels the evolution of resistance more strongly than the evolution of increased sensitivity when therapy is off. This finding

parallels a potential frustration in the management of evolving fisheries stocks, where selection for younger age and smaller size at maturity (which ultimately reduce yield) imposed by harvest is much faster and more intense than the reverse process where natural selection for older age and larger size at maturity during harvest moratoriums may restore a stock (Allendorf et al., 2008).

The second main observation concerns context with a sensitive and a resistant subpopulation of cancer cells, where resistance in the resistant subpopulation evolves. Here, AT always outperforms the MTD protocol by large margins. Regardless of AT or MTD therapies, time to treatment failure (progression) declines as evolutionary speed increases. However the proportional benefit of AT over MTD increases with high evolutionary speeds. The model predicted a 5% improvement with AT at a very low evolutionary speed and about a 55% improvement with a very high evolutionary speed. This is again independent of whether or not there is a cost of resistance. The absolute TTP is for most model parameterization shortest when there is no cost of resistance, followed by a cost of resistance in $r$, and longest when the cost of resistance effects $K$. Whether AT or MTD, TTP is fastest when there is no cost of resistance and slowest when the cost of resistance effects $K$. The greater effectiveness of AT over MTD with a polymorphic population derives from two sources of evolution. First, the resistant subpopulation of cancer cells evolves similarly to that of a monomorphic population. Second, there is a change in the frequency of the sensitive (non-evolving) and resistant subpopulations with each cycle of switching therapy on and off. This reservoir of sensitive cells already has the optimal resistance strategy of $u_S = 0$ when therapy stops, and therefore their population rebounds faster than if one is waiting for the resistant cells to evolve greater sensitivity. And when therapy resumes they are least able to survive.

To summarize, in determining the superiority of AT over MTD it matters most whether the cancer population is monomorphic or polymorphic. While a slower evolutionary speed renders both AT and MTD more effective, the relative superiority of AT decreases with evolutionary speed in the monomorphic population, and increases with evolutionary speed in the polymorphic population. Overall, evolutionary speed matters more in the context of the polymorphic cancer population than the monomorphic one. Interestingly the impact of the cost of resistance on the superiority of AT is not that large. While having a cost of resistance increases TTP regardless of AT and MTD, the superiority of AT over MTD is generally greatest when there is no cost of resistance. This result was anticipated by others who noted that AT can be effective relative to MTD even when there is no cost of resistance (Strobl et al., 2021; Viossat and Noble, 2021).

## 6.2. Relation of Our Models and Results to Those Related to Managing/Controlling Other Evolving Systems

Models analyzed within this paper are generic in the sense that they can frame any situation where actions of a human are aimed at controlling, containing, or preserving biological systems evolving according to natural selection, such as fisheries (Salvioli et al., 2021), pest systems (Brown and Staňková, 2017; Cunningham, 2019), or parasites systems (Hastings and d'Alessandro, 2000; Bushman et al., 2018; Scott et al., 2018). In such systems, the human player impacts the eco-evolutionary response in the biological system under management, while the biological system adapts to the human's actions. The control corresponding to the AT in cancer treatment can be considered as the simplest form of evolutionary control of evolving biological systems, where maximum exploitation/control is paused when the population of the biological systems shrinks to half of its initial size. Similarly as we discussed for our model, the impact of evolution of resistance in other biological systems, such as in bacteria and virus strains, will depend on the speed of this evolution (Sandoval-Motta and Aldana, 2016). Our results imply that when the biological system is monomorphic and the evolutionary trait is quantitative, there is little difference between an adaptive and continuous-mortality approach. However, when the biological system is polymorphic, adaptive approaches may be more effective at prolonging the evolution of resistance to the source of mortality than is maintaining a constant preservation/exploitation strategy all the time.

The applicability and desirability of this outcome for wild populations depend on the objectives of management. In the case of harvested (fished, hunted) populations, it seems unlikely that continuous harvest (the MTD approach) could select for a resistant population that "progresses" to immunity from the mortality source and thus to high population density (like a cancer cell population escaping therapy) before harvest simply extirpates the population. In this case, an "AT" approach with harvest moratoria simply allows ecological population recovery with potentially minimal impact on the selective landscape and population genetic variation. In the case of selection inadvertently imposed by human activities (e.g., by urbanization), rapid evolution and short "TTP" of imperiled native plant and animal populations is desirable from a conservation standpoint, and a continuous "MTD" context is unavoidable given the persistent nature of the selective agent (e.g., unrelenting land development, pollution in the Anthropocene). The wild population context most informed by our contrast of mono- and polymorphic cancer populations is arguably that of pests and pathogens where, as with adaptive cancer therapy, human managers directly regulate the application of the mortality source (biocidal agent) and a management goal is learning to live with chronic, small, treatment-sensitive pest populations (Wrubel and Gressel, 1994). While not feasible for cancer as yet, the benefits of AT can be achieved in space as is done in some agricultural applications, where the pesticide is applied to some fraction of the field (e.g., 3/4) while a bit of field is left untreated so as to maintain a sensitive population (Denholm and Rowland, 1992; Weisz et al., 1996).

## 6.3. Other Evolutionary Approaches for Managing Evolving Resources

In this article we considered a classical protocol for AT introduced by Zhang et al. (2017). Other types of evolutionary

therapy, i.e., therapy anticipating and steering evolution of treatment-induced resistance, exist, many of which are inspired by ecological literature. Such include double-bind, evolutionary gambit, extinction therapies, therapies targeting aggregation effects, or those aiming at tumor stabilization (Gatenby et al., 2009a, 2019; Brown and Staňková, 2017; Cunningham et al., 2020). A double-bind therapy works if in evolving resistance to drug A the cancer cells become more susceptible to drug B and vice-versa (Gatenby et al., 2009a; Basanta et al., 2012). An evolutionary gambit, or Sucker's gambit, involves applying a treatment that, while promoting or not inhibiting tumor growth, sets up an evolutionary trap by selecting for cancer cells more susceptible to the planned therapy (Merlo et al., 2006; Basanta and Anderson, 2013). While not yet tested, therapies may be able to exploit aggregation effects. Much of the time, cancer cells, by detoxifying a therapy or by providing protective groups, provide a public good to each other. But, there is a flip side. If the resistance strategy involves pumping the still toxic agent into the interstitial fluid, then the cancer cells may engage in a game of "hot potato"; each is under selection to escalate the number of pumps or other membrane mechanisms for removing the toxin. It becomes an evolutionary race that is driven by natural selection but harmful to the entire population of cancer cells (Brown and Staňková, 2016).

In extinction therapy, the idea is to use a first strike set of drugs, radiation and/or surgery to render the remaining cancer cell population fragmented and dispersed in what may be undetectably small remnant populations. Rather than continuing these therapies, one immediately switches to second strike therapies aimed at "kicking the cancer while it's down." The goal is to apply and then swiftly change up therapies before the cancer cells can evolve resistance. The second strike therapies may be older, out-of-favor drugs (Dinić et al., 2020) or part of the broader pool of current therapy options. They just need to be effective at continuing to drive the cancer cell populations to smaller and smaller sizes, and ideally offer diverse modes of action. The triple-drug overkill strategy used effectively for HIV treatment anticipates this sort of first-strike—second-strike therapies. Termed "pyramiding" (Palumbi, 2001), similar overkill strategies are utilized in pesticide management programs. These treatment strategies apply the maximum application possible, as the intent is curative or the elimination of the pest. If the curative intent is unrealistic, focusing on maintaining the disease/pest burden at acceptable levels becomes a better strategy and should involve less treatment ("minimum effective dose," Cunningham, 2019).

Some of these evolutionary strategies have been successful at managing other evolving biological systems other than cancer. For example, Guiver et al. (2016) controlled pests by cycling between different pest management strategies. Acheson and Gardner (2014) successfully adopted evolutionary harvesting of lobsters, by releasing the smallest and the largest individuals. When targeting antibiotic resistance, Kim et al. (2014), Yoshida et al. (2017), and Imamovic and Sommer (2013) successfully slowed the evolution of a resistant strain by cycling between certain combinations of antibiotics.

It is important to note that an evolutionarily informed management strategy must consider and constantly update the eco-evolutionary state of the system under management. Hence, management differs from metronomic cancer treatments where predefined periods of treatment are punctuated by predefined drug holidays (Cunningham et al., 2018). The failure of metronomic regimens can be seen outside of the cancer domain. For example, when targeting viruses, applying more frequent but shorter predefined antibiotic courses favors the resistant strains (Blanquart et al., 2018). The most advanced evolutionary therapy optimizes treatment objectives with respect to the predefined treatment goals, adopting the Stackelberg evolutionary game theory approach (Staňková et al., 2019; Salvioli, 2020; Wölfl et al., 2020; Salvioli et al., 2021). If successful, this approach leads to the best possible evolutionary control.

## 6.4. Resistance as a Qualitative Trait

In our polymorphic model, the resistant subpopulation carries a "hurdle" of evolvability, as it starts at initially low resistance levels and may evolve to a high resistance rate. In that sense, a limiting case of our polymorphic model is the situation where the resistance trait evolves to the fitness maximum and resistance becomes a qualitative trait. There are multiple cancer models considering such a situation. For example, models of metastatic castrate-resistant prostate cancer consider three different types of cancer cells differing in their sensitivity to androgen deprivation and abiraterone acetate (Cunningham et al., 2011, 2018, 2020; You et al., 2017; Zhang et al., 2017). For these models, AT extends patient TTP. Similarly, Strobl et al. (2021) and Viossat and Noble (2021) demonstrated that AT extends TTP even without a resistance cost.

## 6.5. Different Forms of Cost of Resistance and Its Management

Typically, when a cost of resistance in cancer cells, pests, viruses or bacteria is considered/studied, it is assumed that resistance comes at a cost such as a decreased maximum growth rate. Xu et al. (2014), Kam et al. (2015) and Gallaher et al. (2018) showed that doxorubicin-resistant MCF-7/Dox breast cancer cells replicate slower compared to their sensitive counterparts. Almost a decade earlier, Andersson and Hughes (2010) demonstrated cost of resistance in laboratory experiments with different bacterial strains.

Salvioli (2020) considered how the cost of resistance decreases carrying capacity of cancer cells, focusing on equilibrium behavior instead of on the transient dynamics. Independently whether the cost of resistance impacts growth rate or carrying capacity of the evolving population, it becomes essential to introduce a resistance management plan that defines how resistance can be targeted (Staňková et al., 2019). Levy and Marshall (2004) focused on managing microbial resistance. They proposed tracking the frequency of resistant bacterial strains among patients, isolating hospitalized individuals with potentially dangerous resistant bacteria, and providing such patients with new therapeutic approaches to specifically target resistance. While the second suggestion may be difficult to follow when targeting treatment-induced resistance in cancer, the first

and third suggestions cannot be followed as usefully in the treatment of cancer.

On the other hand, while in some cancers, cells may mutate from being resistant to being sensitive, reversibility of evolutionary traits is expected to be slow or non-existent elsewhere, as already shown for antibiotics (Andersson and Hughes, 2010), fish (Enberg et al., 2009), and pests (Mallet, 1989).

### 6.6. Future Research

In this research, we established a modeling protocol and partially answered the question "What is the best treatment choice based on the speed of evolution of resistance in cancer cells?", listed as one the key questions of ecology and evolution of cancer (Dujon et al., 2020). We also identified the importance of knowing whether the resistance mechanism manifests as a quantitative trait in a monomorphic population or as a resistant vs. sensitive polymorphic population.

Here we focused on the impact of different modeling assumptions on the time to progression of MTD when compared to that of adaptive therapy with a pre-specified schedule. However, future work can include optimizing the treatment schedule for the models introduced here with respect to prespecified criteria, as has been done in other works (Martin et al., 1992; Carrére, 2017; Muros et al., 2017; Cunningham et al., 2018, 2020; Almeida et al., 2019; Gluzman et al., 2020; Wölfl et al., 2020).

Analysis of our modeling results are based on tumor burden. In many clinical instances neoadjuvant therapy such as surgery is applied to reduce tumor burden before or after drug exposure. Changing the population density could ultimately impact the better choice in therapeutic strategy. In an ideal situation, neoadjuvant therapy would reduce the tumor burden to a size such that the subsequent treatment is sufficient in removing all cancer cells. Our model can be used to explore different initial population sizes to analyze the consequences of neoadjuvant therapy. Also, we can use our model to explore timing between therapeutic strategies.

Our model can further be expanded to consider other tumor constituents related to tumor growth and progression. For example, the tumor microenvironment consists of fibroblasts, endothelial cells, immune cells, and non-cellular components that are not explicitly included in our model. Parameters in the model encompass what these cells and factors contribute to overall tumor dynamics. For example, cancer associated fibroblasts (CAFs) and tumor associated macrophages (TAMs) secrete proteins that promote angiogenesis and therefore could be included in the carrying capacity as the abundance of resources (blood vessels) place limits on tumor size. The parameters used in this work were chosen for illustrative purposes. Future research will focus on validating the model using patient data and on tailoring the model to describe specific types of cancer. Stochastic elements, such as mutation of cancer cells into cells of different type, may be included in our model as well. Moreover, future research on collectively evaluating evolutionary speed, the contexts of resistance evolution, and the role of costs of resistance, might profitably investigate how alternative evolutionary therapies, such as those stabilizing the tumor burden (Cunningham et al., 2020), double-bind therapies (Gatenby et al., 2009a), or those motivated by theories of extinction (Gatenby et al., 2019) compare to standard of care and the AT analyzed in this paper.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

MP and MS carried out modeling. DL and CR put the results into the context of the large body of the literature. JB and KS managed the project and organized its different sub-tasks. All authors contributed to writing the manuscript and its final editing.

## FUNDING

This research was supported by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska- Curie grant agreement No's 690817 and 955708, the Dutch National Foundation (NWO) Grant number OCENW.KLEIN.277, the James S. McDonnell Foundation grant, Cancer therapy: Perturbing a complex adaptive system, a V Foundation grant, NIH/National Cancer Institute (NCI) R01CA170595, Application of Evolutionary Principles to Maintain Cancer Control (PQ21), NIH/NCI U54CA143970-05 Physical Science Oncology Network (PSON) Cancer as a complex adaptive system, Alfred P. Sloan Foundation University Center of Exemplary Mentoring (UCEM) #G-2017-9717, and NSF Florida-Georgia Louis Alliance for Minority Participation (FGLSAMP) Bridge to the Doctorate Activity HRD #1906518. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.681121/full#supplementary-material

## REFERENCES

Acheson, J., and Gardner, R. (2014). Fishing failure and success in the gulf of maine: lobster and groundfish management. *Marit. Stud.* 13:8. doi: 10.1186/2212-9790-13-8

Aktipis, C. A., Kwan, V. S., Johnson, K. A., Neuberg, S. L., and Maley, C. C. (2011). Overlooking evolution: a systematic analysis of cancer relapse and therapeutic resistance research. *PLoS ONE* 6(11):e26100. doi: 10.1371/journal.pone.0026100

Alberti, M. (2016). *Cities that Think Like Planets: Complexity, Resilience, and Innovation in Hybrid Ecosystems*. Seattle, WA: University of Washington Press.

Allendorf, F. W., England, P. R., Luikart, G., Ritchie, P. A., and Ryman, N. (2008). Genetic effects of harvest on wild animal populations. *Trends Ecol. Evol.* 23, 327–337. doi: 10.1016/j.tree.2008.02.008

Allendorf, F. W., and Hard, J. J. (2009). Human-induced evolution caused by unnatural selection through harvest of wild animals. *Proc. Natl. Acad. Sci. U.S.A.* 106(Suppl 1), 9987–9994. doi: 10.1073/pnas.0901069106

Almeida, L., Bagnerini, P., Fabrini, G., Hughes, B. D., and Lorenzi, T. (2019). Evolution of cancer cell populations under cytotoxic therapy and treatment optimisation: insight from a phenotype-structured model. *ESAIM Mathemat. Modell. Numer. Anal.* 53, 1157–1190. doi: 10.1051/m2an/2019010

Andersson, D. I., and Hughes, D. (2010). Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat. Rev. Microbiol.* 8, 260–271. doi: 10.1038/nrmicro2319

Bailey, J., and Conolly, A. (2000). Prize-winners to pariahs-a history of Japanese knotweed sl (polygonaceae) in the British Isles. *Watsonia* 23, 93–110. Available online at: http://archive.bsbi.org.uk/Wats23p93.pdf

Bailey, J. P., Bimová, K., and Mandák, B. (2009). Asexual spread versus sexual reproduction and evolution in Japanese knotweed sl sets the stage for the "battle of the clones". *Biol. Invas.* 11, 1189–1203. doi: 10.1007/s10530-008-9381-4

Banta, J. A., and Richards, C. L. (2018). Quantitative epigenetics and evolution. *Heredity* 121, 210–224. doi: 10.1038/s41437-018-0114-x

Barrett, R. D., and Schluter, D. (2008). Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23, 38–44. doi: 10.1016/j.tree.2007.09.008

Basanta, D., and Anderson, A. R. (2013). Exploiting ecological principles to better understand cancer progression and treatment. *Interface Focus* 3:20130020. doi: 10.1098/rsfs.2013.0020

Basanta, D., Gatenby, R. A., and Anderson, A. R. (2012). Exploiting evolution to treat drug resistance: combination therapy and the double bind. *Mol. Pharmaceut.* 9, 914–921. doi: 10.1021/mp200458e

Baucom, R. S. (2019). Evolutionary and ecological insights from herbicide-resistant weeds: what have we learned about plant adaptation, and what is left to uncover? *N. Phytol.* 223, 68–82. doi: 10.1111/nph.15723

Bayer, P., Gatenby, R. A., McDonald, P. H., Derek, K., Duckett, R., Staňková, K., et al. (2021). Coordination games in cancer. submitted. *bioRxiv*. doi: 10.1101/2021.06.22.449436

Beerling, D. J., Bailey, J. P., and Conolly, A. P. (1994). Fallopia japonica (houtt.) ronse decraene. *J. Ecol.* 82, 959–979. doi: 10.2307/2261459

Blanquart, F., Lehtinen, S., Lipsitch, M., and Fraser, C. (2018). The evolution of antibiotic resistance in a structured host population. *J. R. Soc. Interface* 15:20180040. doi: 10.1098/rsif.2018.0040

Bock, D. G., Caseys, C., Cousens, R. D., Hahn, M. A., Heredia, S. M., Hübner, S., et al. (2015). What we still don't know about invasion genetics. *Mol. Ecol.* 24, 2277–2297. doi: 10.1111/mec.13032

Bonduriansky, R., and Day, T. (2009). Nongenetic inheritance and its evolutionary implications. *Annu. Rev. Ecol. Evol. Systemat.* 40, 103–125. doi: 10.1146/annurev.ecolsys.39.110707.173441

Bonduriansky, R., and Day, T. (2018). *Extended Heredity: A New Understanding of Inheritance and Evolution.* Princeton, NJ: Princeton University Press.

Borowy, D., and Swan, C. M. (2020). A multi-trait comparison of an urban plant species pool reveals the importance of intraspecific trait variation and its influence on distinct functional responses to soil quality. *Front. Ecol. Evol.* 8:68. doi: 10.3389/fevo.2020.00068

Brown, J., Cunningham, J., and Gatenby, R. (2016). Aggregation effects and population-based dynamics as a source of therapy resistance in cancer. *IEEE Trans. Biomed. Eng.* 64, 512–518. doi: 10.1109/TBME.2016.2623564

Brown, J., and Staňková, K. (2017). Game theory as a conceptual framework for managing insect pests. *Curr. Opin. Insect Sci.* 21, 26–32. doi: 10.1016/j.cois.2017.05.007

Buckley, J., and Bridle, J. R. (2014). Loss of adaptive variation during evolutionary responses to climate change. *Ecol. Lett.* 17, 1316–1325. doi: 10.1111/ele.12340

Bushman, M., Antia, R., Udhayakumar, V., and de Roode, J. C. (2018). Within-host competition can delay evolution of drug resistance in malaria. *PLoS Biol.* 16:e2005712. doi: 10.1371/journal.pbio.2005712

Cairns, J. (1975). Mutation selection and the natural history of cancer. *Nature* 255, 197–200. doi: 10.1038/255197a0

Carrére, C. (2017). Optimization of an *in vitro* chemotherapy to avoid resistant tumours. *J. Theoret. Biol.* 413, 24–33. doi: 10.1016/j.jtbi.2016.11.009

Chabner, B. A., and Roberts, T. G. (2005). Chemotherapy and the war on cancer. *Nat. Rev. Cancer* 5, 65–72. doi: 10.1038/nrc1529

Chapuis, E., Lamy, T., Pointier, J.-P., Juillet, N., Ségard, A., Jarne, P., et al. (2017). Bioinvasion triggers rapid evolution of life histories in freshwater snails. *Am. Nat.* 190, 694–706. doi: 10.1086/693854

Cheptou, P.-O., Carrue, O., Rouifed, S., and Cantarel, A. (2008). Rapid evolution of seed dispersal in an urban environment in the weed crepis sancta. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3796–3799. doi: 10.1073/pnas.0708446105

Coffey, D. S. (1998). Self-organization, complexity and chaos: the new biology for medicine. *Nat. Med.* 4, 882–885. doi: 10.1038/nm0898-882

Colautti, R. I., and Lau, J. A. (2015). Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Mol. Ecol.* 24, 1999–2017. doi: 10.1111/mec.13162

Coldsnow, K. D., Mattes, B. M., Hintz, W. D., and Relyea, R. A. (2017). Rapid evolution of tolerance to road salt in zooplankton. *Environ. Pollut.* 222, 367–373. doi: 10.1016/j.envpol.2016.12.024

Collins, J. P., Kinzig, A., Grimm, N. B., Fagan, W. F., Hope, D., Wu, J., et al. (2000). A new urban ecology: modeling human communities as integral parts of ecosystems poses special problems for the development and testing of ecological theory. *Am. Sci.* 88, 416–425. doi: 10.1511/2000.5.416

Comont, D., Lowe, C., Hull, R., Crook, L., Hicks, H. L., Onkokesung, N., et al. (2020). Evolution of generalist resistance to herbicide mixtures reveals a trade-off in resistance management. *Nat. Commun.* 11, 1–9. doi: 10.1038/s41467-020-18079-3

Conover, D. O. (2000). Darwinian fishery science. *Mar. Ecol. Prog. Ser.* 208, 303–307. Available online at: https://www.jstor.org/stable/24863828

Conover, D. O., and Munch, S. B. (2002). Sustaining fisheries yields over evolutionary time scales. *Science* 297, 94–96. doi: 10.1126/science.1074085

Cortijo, S., Wardenaar, R., Colomé-Tatché, M., Gilly, A., Etcheverry, M., Labadie, K., et al. (2014). Mapping the epigenetic basis of complex traits. *Science* 343, 1145–1148. doi: 10.1126/science.1248127

Cunningham, J. (2019). A call for integrated metastatic management. *Nat. Ecol. Evol.* 3, 996–998. doi: 10.1038/s41559-019-0927-x

Cunningham, J. J., Brown, J. S., Gatenby, R. A., and Staňková, K. (2018). Optimal control to develop therapeutic strategies for metastatic castrate resistant prostate cancer. *J. Theoret. Biol.* 459, 67–78. doi: 10.1016/j.jtbi.2018.09.022

Cunningham, J. J., Gatenby, R. A., and Brown, J. S. (2011). Evolutionary dynamics in cancer therapy. *Mol. Pharmaceut.* 8, 2094–2100. doi: 10.1021/mp2002279

Cunningham, J. J., Thuijsman, F., Peeters, R., Viossat, Y., Brown, J. S., Gatenby, R. A., et al. (2020). Optimal control to reach eco-evolutionary stability in metastatic castrate resistant prostate cancer. *PLoS ONE* 15:e0243386. doi: 10.1371/journal.pone.0243386

De Groot, A. E., Roy, S., Brown, J. S., Pienta, K. J., and Amend, S. R. (2017). Revisiting seed and soil: examining the primary tumor and cancer cell foraging in metastasis. *Mol. Cancer Res.* 15, 361–370. doi: 10.1158/1541-7786.MCR-16-0436

Denholm, I., and Rowland, M. (1992). Tactics for managing pesticide resistance in arthropods: theory and practice. *Annu. Rev. Entomol.* 37, 91–112. doi: 10.1146/annurev.en.37.010192.000515

Diamond, S. E., Chick, L., Perez, A., Strickler, S. A., and Martin, R. A. (2017). Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. *Biol. J. Linnean Soc.* 121, 248–257. doi: 10.1093/biolinnean/blw047

Dieckmann, U., and Law, R. (1996). The dynamical theory of coevolution: a derivation from stochastic ecological processes. *J. Math. Biol.* 34, 579–612. doi: 10.1007/s002850050022

Dinić, J., Efferth, T., Garcia-Sosa, A. T., Grahovac, J., Padrón, J. M., Pajeva, I., et al. (2020). Repurposing old drugs to fight multidrug resistant cancers. *Drug Resist.* 52:100713. doi: 10.1016/j.drup.2020.100713

Dlugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A., and Gillette, H. D. (2015). The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Mol. Ecol.* 24, 2095–2111. doi: 10.1111/mec.13183

Duesberg, P., Rausch, C., Rasnick, D., and Hehlmann, R. (1998). Genetic instability of cancer cells is proportional to their degree of aneuploidy. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13692–13697. doi: 10.1073/pnas.95.23.13692

Dujon, A. M., Aktipis, A., Alix-Panabiéres, C., Amend, S. R., Boddy, A. M., Brown, J. S., et al. (2020). Identifying key questions in the ecology and evolution of cancer. *Evol. Appl.* 14, 877–892. doi: 10.1111/eva.13190

Enberg, K., Jørgensen, C., Dunlop, E. S., Heino, M., and Dieckmann, U. (2009). Implications of fisheries-induced evolution for stock rebuilding

and recovery. *Evol. Appl.* 2, 394–414. doi: 10.1111/j.1752-4571.2009.00077.x

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., and Facon, B. (2016). Is there a genetic paradox of biological invasion? *Annu. Rev. Ecol. Evol. Syst.* 47, 51–72. doi: 10.1146/annurev-ecolsys-121415-032116

Feinberg, A. P., Ohlsson, R., and Henikoff, S. (2006). The epigenetic progenitor origin of human cancer. *Nat. Rev. Genet.* 7, 21–33. doi: 10.1038/nrg1748

Fisher, R. (1930). *The Genetical Theory of Natural Selection*. Oxford, UK: Clarendon Press. doi: 10.5962/bhl.title.27468

Franks, S. J., Sim, S., and Weis, A. E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc. Natl. Acad. Sci. U.S.A.* 104, 1278–1282. doi: 10.1073/pnas.0608379104

Fritts, T. H., and Rodda, G. H. (1998). The role of introduced species in the degradation of island ecosystems: a case history of guam. *Annu. Rev. Ecol. Syst.* 29, 113–140. doi: 10.1146/annurev.ecolsys.29.1.113

Fugére, V., and Hendry, A. P. (2018). Human influences on the strength of phenotypic selection. *Proc. Natl. Acad. Sci. U.S.A.* 115, 10070–10075. doi: 10.1073/pnas.1806013115

Galbreath, R., and Brown, D. (2004). The tale of the lighthouse-keeper's cat: discovery and extinction of the Stephens Island wren (Traversia lyalli). *Notornis* 51, 193–200. Available online at: http://notornis.osnz.org.nz/system/files/Notornis_51_4_193.pdf

Gallaher, J., Enriquez-Navas, P., Luddy, K., Gatenby, R., and Anderson, A. (2018). Spatial heterogeneity and evolutionary dynamics modulate time to recurrence in continuous and adaptive cancer therapies. *Cancer Res.* 78, 2127–2139. doi: 10.1158/0008-5472.CAN-17-2649

Gatenby, R. A. (2009). A change of strategy in the war on cancer. *Nature* 459:508. doi: 10.1038/459508a

Gatenby, R. A., Brown, J., and Vincent, T. (2009a). Lessons from applied ecology: cancer control using an evolutionary double bind. *Cancer Res.* 69, 7499–7502. doi: 10.1158/0008-5472.CAN-09-1354

Gatenby, R. A., and Brown, J. S. (2020a). The evolution and ecology of resistance in cancer therapy. *Cold Spring Harbor Perspect. Med.* 10:a040972. doi: 10.1101/cshperspect.a040972

Gatenby, R. A., and Brown, J. S. (2020b). Integrating evolutionary dynamics into cancer therapy. *Nat. Rev. Clin. Oncol.* 17, 675–686. doi: 10.1038/s41571-020-0411-1

Gatenby, R. A., Silva, A. S., Gillies, R. J., and Frieden, B. R. (2009b). Adaptive therapy. *Cancer Res.* 69, 4894–4903. doi: 10.1158/0008-5472.CAN-08-3658

Gatenby, R. A., Zhang, J., and Brown, J. S. (2019). First strike-second strike strategies in metastatic cancer: lessons from the evolutionary dynamics of extinction. *Cancer Res.* 79, 3174–3177. doi: 10.1158/0008-5472.CAN-19-0807

Geerts, A., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feuchtmayr, H., Atkinson, D., et al. (2015). Rapid evolution of thermal tolerance in the water flea daphnia. *Nat. Clim. Change* 5, 665–668. doi: 10.1038/nclimate2628

Gerber, E., Krebs, C., Murrell, C., Moretti, M., Rocklin, R., and Schaffner, U. (2008). Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in european riparian habitats. *Biol. Cons.* 141, 646–654. doi: 10.1016/j.biocon.2007.12.009

Geritz, S. A., Mesze, G., Metz, J. A., et al. (1998). Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. *Evol. Ecol.* 12, 35–57. doi: 10.1023/A:1006554906681

Gerstung, M., Jolly, C., Leshchiner, I., Dentro, S. C., Gonzalez, S., Rosebrock, D., et al. (2020). The evolutionary history of 2,658 cancers. *Nature* 578, 122–128. doi: 10.1038/s41586-019-1907-7

Gluzman, M., Scott, J. G., and Vladimirsky, A. (2020). Optimizing adaptive cancer therapy: dynamic programming and evolutionary game theory. *Proc. R. Soc. B.* 287:20192454. doi: 10.1098/rspb.2019.2454

Greaves, M., and Maley, C. C. (2012). Clonal evolution in cancer. *Nature* 481, 306–313. doi: 10.1038/nature10762

Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., et al. (2008). Global change and the ecology of cities. *Science* 319, 756–760. doi: 10.1126/science.1150195

Grimsby, J. L., Tsirelson, D., Gammon, M. A., and Kesseli, R. (2007). Genetic diversity and clonal vs. sexual reproduction in *Fallopia* spp.(polygonaceae). *Am. J. Bot.* 94, 957–964. doi: 10.3732/ajb.94.6.957

Guiver, C., Edholm, C., Jin, Y., Mueller, M., Powell, J., Rebarber, R., et al. (2016). Simple adaptive control for positive linear systems with applications to pest management. *SIAM J. Appl. Math.* 76, 238–275. doi: 10.1137/140996926

Hairston, N. G. Jr., Ellner, S. P., Geber, M. A., Yoshida, T., and Fox, J. A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.* 8, 1114–1127. doi: 10.1111/j.1461-0248.2005.00812.x

Hanahan, D., and Weinberg, R. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* 100, 57–70. doi: 10.1016/S0092-8674(00)81683-9

Harris, S. E., Munshi-South, J., Obergfell, C., and O'Neill, R. (2013). Signatures of rapid evolution in urban and rural transcriptomes of white-footed mice (*Peromyscus leucopus*) in the New York metropolitan area. *PLoS ONE* 8:e74938. doi: 10.1371/journal.pone.0074938

Hastings, A., and Gross, L. (2012). *Encyclopedia of Theoretical Ecology*. Berkeley: University of California Press. doi: 10.1525/9780520951785

Hastings, I., and d'Alessandro, U. (2000). Modelling a predictable disaster:: the rise and spread of drug-resistant malaria. *Parasitol. Tdy* 16, 340–347. doi: 10.1016/S0169-4758(00)01707-5

Hawkins, N. J., Bass, C., Dixon, A., and Neve, P. (2019). The evolutionary origins of pesticide resistance. *Biol. Rev.* 94, 135–155. doi: 10.1111/brv.12440

Heino, M. (1998). Management of evolving fish stocks. *Can. J. Fish. Aquat. Sci.* 55, 1971–1982. doi: 10.1139/f98-089

Hendry, A., Gotanda, K., and Svensson, E. (2017). Human influences on evolution, and the ecological and societal consequences. *Philos. Trans. R. Soc. Lond. Ser. B Bio. Sci.* 372:1712. doi: 10.1098/rstb.2016.0028

Hendry, A. P., Farrugia, T. J., and Kinnison, M. T. (2008). Human influences on rates of phenotypic change in wild animal populations. *Mol. Ecol.* 17, 20–29. doi: 10.1111/j.1365-294X.2007.03428.x

Hoffmann, A. A., and Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature* 470, 479–485. doi: 10.1038/nature09670

Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D., and Serra, L. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287, 308–309. doi: 10.1126/science.287.5451.308

Imamovic, L., and Sommer, M. O. (2013). Use of collateral sensitivity networks to design drug cycling protocols that avoid resistance development. *Sci. Transl. Med.* 5:204ra132. doi: 10.1126/scitranslmed.3006609

Jablonka, E., and Raz, G. (2009). Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.* 84, 131–176. doi: 10.1086/598822

Jachmann, H., Berry, P., and Imae, H. (1995). Tusklessness in african elephants: a future trend. *Afr. J. Ecol.* 33, 230–235. doi: 10.1111/j.1365-2028.1995.tb00800.x

Johnson, M. T., and Munshi-South, J. (2017). Evolution of life in urban environments. *Science* 358:6363. doi: 10.1126/science.aam8327

Jorgensen, C., Enberg, K., Dunlop, E. S., Arlinghaus, R., Boukal, D. S., Brander, K., et al. (2007). Ecology-managing evolving fish stocks. *Science* 318, 1247–1248. doi: 10.1126/science.1148089

Kadoch, C., and Crabtree, G. R. (2015). Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. *Sci. Adv.* 1:e1500447. doi: 10.1126/sciadv.1500447

Kam, Y., Das, T., Tian, H., Foroutan, P., Ruiz, E., Martinez, G., et al. (2015). Sweat but no gain: inhibiting proliferation of multidrug resistant cancer cells with "ersatzdroges". *Int. J. Cancer* 136, E188–E196. doi: 10.1002/ijc.29158

Keller, E. F. (2014). From gene action to reactive genomes. *J. Physiol.* 592, 2423–2429. doi: 10.1113/jphysiol.2014.270991

Kim, E., Brown, J. S., Eroglu, Z., and Anderson, A. R. A. (2021). Adaptive therapy for metastatic melanoma: predictions from patient calibrated mathematical models. *Cancers* 13:823. doi: 10.3390/cancers13040823

Kim, S., Lieberman, T. D., and Kishony, R. (2014). Alternating antibiotic treatments constrain evolutionary paths to multidrug resistance. *Proc. Natl. Acad. Sci. U.S.A.* 111, 14494–14499. doi: 10.1073/pnas.1409800111

Klironomos, F. D., Berg, J., and Collins, S. (2013). How epigenetic mutations can affect genetic evolution: model and mechanism. *Bioessays* 35, 571–578. doi: 10.1002/bies.201200169

Kopp, M., and Matuszewski, S. (2014). Rapid evolution of quantitative traits: theoretical perspectives. *Evol. Appl.* 7, 169–191. doi: 10.1111/eva.12127

Kronholm, I., Bassett, A., Baulcombe, D., and Collins, S. (2017). Epigenetic and genetic contributions to adaptation in chlamydomonas. *Mol. Biol. Evol.* 34, 2285–2306. doi: 10.1093/molbev/msx166

Kronholm, I., and Collins, S. (2016). Epigenetic mutations can both help and hinder adaptive evolution. *Mol. Ecol.* 25, 1856–1868. doi: 10.1111/mec.13296

Kuester, A., Conner, J. K., Culley, T., and Baucom, R. S. (2014). How weeds emerge: a taxonomic and trait-based examination using united states data. *N. Phytol.* 202, 1055–1068. doi: 10.1111/nph.12698

Kuparinen, A., and Festa-Bianchet, M. (2017). Harvest-induced evolution: insights from aquatic and terrestrial systems. *Philos. Trans. R. Soc. B Biol. Sci.* 372:20160036. doi: 10.1098/rstb.2016.0036

Lambert, A. W., Pattabiraman, D. R., and Weinberg, R. A. (2017). Emerging biological principles of metastasis. *Cell* 168, 670–691. doi: 10.1016/j.cell.2016.11.037

Lankau, R. A. (2011). Rapid evolutionary change and the coexistence of species. *Annu. Rev. Ecol. Evol. Systemat.* 42, 335–354. doi: 10.1146/annurev-ecolsys-102710-145100

Laundré, J. W., Hernández, L., and Altendorf, K. B. (2001). Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, USA. *Can. J. Zool/* 79, 1401–1409. doi: 10.1139/z01-094

Law, R. (2000). Fishing, selection, and phenotypic evolution. *ICES J. Mar. Sci.* 57, 659–668. doi: 10.1006/jmsc.2000.0731

Law, R. (2007). Fisheries-induced evolution: present status and future directions. *Mar. Ecol. Prog. Ser.* 335, 271–277. doi: 10.3354/meps335271

Ledón-Rettig, C. C., Richards, C. L., and Martin, L. B. (2013). Epigenetics for behavioral ecologists. *Behav. Ecol.* 24, 311–324. doi: 10.1093/beheco/ars145

Levy, S. B., and Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 10, S122–S129. doi: 10.1038/nm1145

Lorenzi, T., Chisholm, R. H., and Clairambault, J. (2016). Tracking the evolution of cancer cell populations through the mathematical lens of phenotype-structured equations. *Biol. Direct* 11, 1–17. doi: 10.1186/s13062-016-0143-4

Mallet, J. (1989). The evolution of insecticide resistance: have the insects won? *Trends Ecol. Evol.* 4, 336–340. doi: 10.1016/0169-5347(89)90088-8

Martin, R. B., Fisher, M. E., Minchin, R. F., and Teo, K. L. (1992). Optimal control of tumor size used to maximize survival time when cells are resistant to chemotherapy. *Math. Biosci.* 110, 201–219. doi: 10.1016/0025-5564(92)90038-X

Medway, D. G. (2004). The land bird fauna of Stephens Island, New Zealand in the early 1890s, and the cause of its demise. *Notornis* 51, 201–211. Available online at: http://notornis.osnz.org.nz/system/files/Notornis_51_4_201.pdf

Merlo, L. M., Pepper, J. W., Reid, B. J., and Maley, C. C. (2006). Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer* 6, 924–935. doi: 10.1038/nrc2013

Metz, J. A., Geritz, S. A., Meszéna, G., Jacobs, F. J., and Van Heerwaarden, J. S. (1995). "Adaptive dynamics: a geometrical study of the consequences of nearly faithful reproduction," in *Stochastic and Spatial Structures of Dynamical Systems, Proceedings of the Royal Dutch Academy of Science (KNAW Verhandelingen)* (Amsterdam), 183–231.

Mounger, J., Ainouche, M., Bossdorf, O., Cave-Radet, A., Li, B., Parepa, M., et al. (2021). Epigenetics and the success of invasive plants. *Philos. Trans. R. Soc. B.* 376:20200117. doi: 10.1098/rstb.2020.0117

Müller, G. B. (2017). Why an extended evolutionary synthesis is necessary. *Interface Focus* 7:20170015. doi: 10.1098/rsfs.2017.0015

Muros, F., Maestre, J., You, L., and Stankova, K. (2017). "Model predictive control for optimal treatment in a spatial cancer game," in *Proceedings of the 56th IEEE Conference on Decision and Control* (Melbourne, VIC). doi: 10.1109/CDC.2017.8264481

Nowell, P. (1976). The clonal evolution of tumor cell populations. *Science* 194, 23–28. doi: 10.1126/science.959840

Olsen, E. M., Heino, M., Lilly, G. R., Morgan, M. J., Brattey, J., Ernande, B., et al. (2004). Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature* 428:932. doi: 10.1038/nature02430

Orlando, P., Gatenby, R., and Brown, J. (2012). Cancer treatment as a game: integrating evolutionary game theory into the optimal control of chemotherapy. *Phys. Biol.* 9:065007. doi: 10.1088/1478-3975/9/6/065007

Palumbi, S. R. (2001). Humans as the world's greatest evolutionary force. *Science* 293, 1786–1790. doi: 10.1126/science.293.5536.1786

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., et al. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* 399, 579–583. doi: 10.1038/21181

Paun, O., Verhoeven, K. J., and Richards, C. L. (2019). Opportunities and limitations of reduced representation bisulfite sequencing in plant ecological epigenomics. *N. Phytol.* 221, 738–742. doi: 10.1111/nph.15388

Pepper, J. W., Scott Findlay, C., Kassen, R., Spencer, S. L., and Maley, C. C. (2009). Cancer research meets evolutionary biology. *Evol. Appl.* 2, 62–70. doi: 10.1111/j.1752-4571.2008.00063.x

Perkins, J. H. (1980). Boll weevil eradication. *Science* 207, 1044–1050. doi: 10.1126/science.207.4435.1044

Pienta, K. J., Hammarlund, E. U., Axelrod, R., Amend, S. R., and Brown, J. S. (2020). Convergent evolution, evolving evolvability, and the origins of lethal cancer. *Mol. Cancer Res.* 18, 801–810. doi: 10.1158/1541-7786.MCR-19-1158

Pigeon, G., Festa-Bianchet, M., Coltman, D. W., and Pelletier, F. (2016). Intense selective hunting leads to artificial evolution in horn size. *Evol. Appl.* 9, 521–530. doi: 10.1111/eva.12358

Pinsky, M. L., and Palumbi, S. R. (2014). Meta-analysis reveals lower genetic diversity in overfished populations. *Mol. Ecol.* 23, 29–39. doi: 10.1111/mec.12509

Pittendrigh, B., Olds, B., Yoon, K., Lee, S., Sun, W., and Clark, J. (2013). The genomics of human lice: from the genome to the potential for future control strategies. *Pesticide Biochem. Physiol.* 106, 172–176. doi: 10.1016/j.pestbp.2013.03.010

Reed, D. R., Metts, J., Pressley, M., Fridley, B. L., Hayashi, M., Isakoff, M. S., et al. (2020). An evolutionary framework for treating pediatric sarcomas. *Cancer* 126, 2577–2587. doi: 10.1002/cncr.32777

Reznick, D. N., Losos, J., and Travis, J. (2019). From low to high gear: there has been a paradigm shift in our understanding of evolution. *Ecol. Lett.* 22, 233–244. doi: 10.1111/ele.13189

Richards, C. L., Alonso, C., Becker, C., Bossdorf, O., Bucher, E., Colomé-Tatché, M., et al. (2017). Ecological plant epigenetics: evidence from model and non-model species, and the way forward. *Ecol. Lett.* 20, 1576–1590. doi: 10.1101/130708

Richards, C. L., Bossdorf, O., Muth, N. Z., Gurevitch, J., and Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* 9, 981–993. doi: 10.1111/j.1461-0248.2006.00950.x

Richards, C. L., Bossdorf, O., and Pigliucci, M. (2010). What role does heritable epigenetic variation play in phenotypic evolution? *Bioscience* 60, 232–237. doi: 10.1525/bio.2010.60.3.9

Richards, C. L., and Pigliucci, M. (2020). Epigenetic inheritance. A decade into the extended evolutionary synthesis. *Paradigmi* 38, 463–494. doi: 10.30460/99624

Richards, C. L., Schrey, A. W., and Pigliucci, M. (2012). Invasion of diverse habitats by few japanese knotweed genotypes is correlated with epigenetic differentiation. *Ecol. Lett.* 15, 1016–1025. doi: 10.1111/j.1461-0248.2012.01824.x

Richards, C. L., Walls, R. L., Bailey, J. P., Parameswaran, R., George, T., and Pigliucci, M. (2008). Plasticity in salt tolerance traits allows for invasion of novel habitat by Japanese knotweed sl (Fallopia japonica and f.× bohemica, polygonaceae). *Am. J. Bot.* 95, 931–942. doi: 10.3732/ajb.2007364

Robinson, D. A., and Enright, M. C. (2003). Evolutionary models of the emergence of methicillin-resistant staphylococcus aureus. *Antimicrob. Agents Chemother.* 47, 3926–3934. doi: 10.1128/AAC.47.12.3926-3934.2003

Rollins, L. A., Richardson, M. F., and Shine, R. (2015). A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Mol. Ecol.* 24, 2264–2276. doi: 10.1111/mec.13184

Rudman, S. M., Kreitzman, M., Chan, K. M., and Schluter, D. (2017). Evosystem services: rapid evolution and the provision of ecosystem services. *Trends Ecol. Evol.* 32, 403–415. doi: 10.1016/j.tree.2017.02.019

Salvioli, M. (2020). *Game theory for improving medical decisions and managing biological systems* (Ph.D. thesis). Politecnico di Milano, Milano, Italy.

Salvioli, M., Dubbeldam, J., Staňková, K., and Brown, J. S. (2021). Fisheries management as a Stackelberg evolutionary game: finding an evolutionarily enlightened strategy. *PLoS ONE* 16:e0245255. doi: 10.1371/journal.pone.0245255

Sandoval-Motta, S., and Aldana, M. (2016). Adaptive resistance to antibiotics in bacteria: a systems biology perspective. *Wiley Interdiscipl. Rev. Syst. Biol. Med.* 8, 253–267. doi: 10.1002/wsbm.1335

Savidge, J. A. (1987). Extinction of an island forest avifauna by an introduced snake. *Ecology* 68, 660–668. doi: 10.2307/1938471

Scott, N., Ataide, R., Wilson, D. P., Hellard, M., Price, R. N., Simpson, J. A., et al. (2018). Implications of population-level immunity for the emergence of artemisinin-resistant malaria: a mathematical model. *Malaria J.* 17, 1–11. doi: 10.1186/s12936-018-2418-y

Selechnik, D., Richardson, M. F., Shine, R., DeVore, J. L., Ducatez, S., and Rollins, L. A. (2019). Increased adaptive variation despite reduced overall genetic diversity in a rapidly adapting invader. *Front. Genet.* 10:1221. doi: 10.3389/fgene.2019.01221

Shipman, M. (2017). *The Boll Weevil War, or How Farmers and Scientists Saved Cotton in the South*. Available online at: https://news.ncsu.edu/2017/05/boll-weevil-war-2017/ (accessed March 12, 2021).

Staňková, K. (2019). Resistance games. *Nat. Ecol. Evol.* 3, 336–337. doi: 10.1038/s41559-018-0785-y

Staňková, K., Brown, J. S., Dalton, W. S., and Gatenby, R. A. (2019). Optimizing cancer treatment using game theory: a review. *JAMA Oncol.* 5, 96–103. doi: 10.1001/jamaoncol.2018.3395

Stapley, J., Santure, A. W., and Dennis, S. R. (2015). Transposable elements as agents of rapid adaptation may explain the genetic paradox of invasive species. *Mol. Ecol.* 24, 2241–2252. doi: 10.1111/mec.13089

Stoltzfus, A. (2017). Why we don't want another "synthesis". *Biol. Direct* 12, 1–12. doi: 10.1186/s13062-017-0194-1

Strobl, M. A., Gallaher, J., West, J., Robertson-Tessi, M., Maini, P. K., and Anderson, A. (2020). Spatial structure impacts adaptive therapy by shaping intra-tumoral competition. *bioRxiv*. doi: 10.1101/2020.11.03.365163

Strobl, M. A., West, J., Viossat, Y., Damaghi, M., Robertson-Tessi, M., Brown, J. S., et al. (2021). Turnover modulates the need for a cost of resistance in adaptive therapy. *Cancer Res.* 81, 1135–1147. doi: 10.1158/0008-5472.CAN-20-0806

Sullivan, A. P., Bird, D. W., and Perry, G. H. (2017). Human behaviour as a long-term ecological driver of non-human evolution. *Nat. Ecol. Evol.* 1, 1–11. doi: 10.1038/s41559-016-0065

Sun, X., Bao, J., and Shao, Y. (2016). Mathematical modeling of therapy-induced cancer drug resistance: connecting cancer mechanisms to population survival rates. *Sci. Rep.* 6, 1–12. doi: 10.1038/srep22498

Sutter, D. A., Suski, C. D., Philipp, D. P., Klefoth, T., Wahl, D. H., Kersten, P., et al. (2012). Recreational fishing selectively captures individuals with the highest fitness potential. *Proc. Natl. Acad. Sci. U.S.A.* 109, 20960–20965. doi: 10.1073/pnas.1212536109

Tewari, S., Leskey, T. C., Nielsen, A. L., Piñero, J. C., and Rodriguez-Saona, C. R. (2014). "Use of pheromones in insect pest management, with special attention to weevil pheromones," in *Integrated Pest Management* ed D. P. Abrol (New York, NY: Elsevier), 141–168. doi: 10.1016/B978-0-12-398529-3.00010-5

Timp, W., and Feinberg, A. P. (2013). Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. *Nat. Rev. Cancer* 13, 497–510. doi: 10.1038/nrc3486

Turner, K. G., Hufbauer, R. A., and Rieseberg, L. H. (2014). Rapid evolution of an invasive weed. *N. Phytol.* 202, 309–321. doi: 10.1111/nph.12634

van Kleunen, M., Bossdorf, O., and Dawson, W. (2018). The ecology and evolution of alien plants. *Annu. Rev. Ecol. Evol. Systemat.* 49, 25–47. doi: 10.1146/annurev-ecolsys-110617-062654

Vandepitte, K., De Meyer, T., Helsen, K., Van Acker, K., Roldán-Ruiz, I., Mergeay, J., et al. (2014). Rapid genetic adaptation precedes the spread of an exotic plant species. *Mol. Ecol.* 23, 2157–2164. doi: 10.1111/mec.12683

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P T Peer Review. J. Formul. Manage.* 40, 277–283.

Vincent, T. L., and Brown, J. S. (2005). *Evolutionary Game Theory, Natural Selection, and Darwinian Dynamics*. New York, NY: Cambridge University Press. doi: 10.1017/CBO9780511542633

Viossat, Y., and Noble, R. (2021). A theoretical analysis of tumour containment. *Nat. Ecol. Evol.* 5, 826–835. doi: 10.1038/s41559-021-01428-w

Weiner, J. (1995). *The Beak of the Finch: A Story of Evolution in Our Time*. New York, NY: Vintage Books.

Weisz, R., Fleischer, S., and Smilowitz, Z. (1996). Site-specific integrated pest management for high-value crops: impact on potato pest management. *J. Econ. Entomol.* 89, 501–509. doi: 10.1093/jee/89.2.501

Wellems, T. E., and Plowe, C. V. (2001). Chloroquine-resistant malaria. *J. Infect. Dis.* 184, 770–776. doi: 10.1086/322858

West, J., Ma, Y., and Newton, P. K. (2018). Capitalizing on competition: an evolutionary model of competitive release in metastatic castration resistant prostate cancer treatment. *J. Theoret. Biol.* 455, 249–260. doi: 10.1016/j.jtbi.2018.07.028

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Systemat.* 20, 249–278. doi: 10.1146/annurev.es.20.110189.001341

Whitney, K. D., and Gabler, C. A. (2008). Rapid evolution in introduced species, "invasive traits" and recipient communities: challenges for predicting invasive potential. *Divers. Distribut.* 14, 569–580. doi: 10.1111/j.1472-4642.2008.00473.x

Wiles, G. J., Bart, J., Beck, R. E. Jr., and Aguon, C. F. (2003). Impacts of the brown tree snake: patterns of decline and species persistence in Guam's Avifauna. *Conserv. Biol.* 17, 1350–1360. doi: 10.1046/j.1523-1739.2003.01526.x

Wölfl, B., te Rietmole, H., Salvioli, M., Kaznatchev, A., Thuijsman, F., Brown, J. S., et al. (2020). The contribution of evolutionary game theory to understanding and treating cancer. *medRxiv*. doi: 10.1101/2020.12.02.20241703

Wrubel, R. P., and Gressel, J. (1994). Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study. *Weed Technol.* 8, 635–648. doi: 10.1017/S0890037X00039828

Xu, H. B., Shen, Z. L., Fu, J., and Xu, L. Z. (2014). Reversal of doxorubicin resistance by guggulsterone of commiphora mukul *in vivo*. *Phytomedicine* 21, 1221–1229. doi: 10.1016/j.phymed.2014.06.003

Yeh, P. J. (2004). Rapid evolution of a sexually selected trait following population establishment in a novel habitat. *Evolution* 58, 166–174. doi: 10.1111/j.0014-3820.2004.tb01583.x

Yoshida, M., Reyes, S. G., Tsuda, S., Horinouchi, T., Furusawa, C., and Cronin, L. (2017). Time-programmable drug dosing allows the manipulation, suppression and reversal of antibiotic drug resistance *in vitro*. *Nat. Commun.* 8, 1–11. doi: 10.1038/ncomms15589

You, L., Brown, J. S., Thuijsman, F., Cunningham, J. J., Gatenby, R. A., Zhang, J., et al. (2017). Spatial vs. non-spatial eco-evolutionary dynamics in a tumor growth model. *J. Theoret. Biol.* 435, 78–97. doi: 10.1016/j.jtbi.2017.08.022

You, L., von Knobloch, M., Lopez, T., Peschen, V., Radcliffe, S., Koshy Sam, P., et al. (2019). Including blood vasculature into a game-theoretic model of cancer dynamics. *Games* 10:13. doi: 10.3390/g10010013

Zhang, J., Cunningham, J. J., Brown, J. S., and Gatenby, R. A. (2017). Integrating evolutionary dynamics into treatment of metastatic castrate-resistant prostate cancer. *Nat. Commun.* 8:1816. doi: 10.1038/s41467-017-01968-5

Zhang, Y.-Y., Parepa, M., Fischer, M., and Bossdorf, O. (2016). Epigenetics of colonizing species? a study of Japanese knotweed in Central Europe. *Invas. Genet.* 328–340. doi: 10.1002/9781119072799.ch19

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frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 17 August 2021
doi: 10.3389/fevo.2021.673082

# Estrogen as an Essential Resource and the Coexistence of $ER^+$ and $ER^-$ Cancer Cells

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Diagnosis of estrogen sensitivity in breast cancer is largely predicated on the ratio of $ER^+$ and $ER^-$ cancer cells obtained from biopsies. Estrogen is a growth factor necessary for cell survival and division. It can also be thought of as an essential resource that can act in association with other nutrients, glucose, glutamine, fatty acids, amino acids, etc. All of these nutrients, collectively or individually, may limit the growth of the cancer cells (Liebig's Law of the Minimum). Here we model estrogen susceptibility in breast cancer as a consumer-resource interaction: $ER^+$ cells require both estrogen and glucose as essential resources, whereas $ER^-$ only require the general resource. The model predicts that when estrogen is the limiting factor, other nutrients may go unconsumed and available at higher levels, thus permitting the invasion of $ER^-$ cells. Conversely, when $ER^-$ cells are less efficient on glucose than $ER^+$ cells, then $ER^-$ cells limited by glucose may be susceptible to invasion by $ER^+$ cells, provided that sufficient levels of estrogen are available. $ER^+$ cells will outcompete $ER^-$ cells when estrogen is abundant, resulting in low concentrations of interstitial glucose within the tumor. In the absence of estrogen, $ER^-$ cells will outcompete $ER^+$ cells, leaving a higher concentration of interstitial glucose. At intermediate delivery rates of estrogen and glucose, $ER^+$ and $ER^-$ cells are predicted to coexist. In modeling the dynamics of cells in the same tumor with different resource requirements, we can apply concepts and terms familiar to many ecologists. These include: resource supply points, $R^*$, ZNGI (zero net growth isoclines), resource depletion, and resource uptake rates. Based on the circumstances favoring $ER^+$ vs. $ER^-$ breast cancer, we use the model to explore the consequences of therapeutic regimens that may include hormonal therapies, possible roles of diet in changing cancer cell composition, and potential for evolutionarily informed therapies. More generally, the model invites the viewpoint that cancer's eco-evolutionary dynamics are a consumer-resource interaction, and that other growth factors such as EGFR or androgens may be best viewed as essential resources within these dynamics.

**Keywords: subsistence levels, estrogen dependence, $ER^+/ER^-$ breast cancer, evolutionary steering, mathematical model, Liebig's Law of the Minimum**

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***Edited by:***
*Frederick R. Adler, The University of Utah, United States*

***Reviewed by:***
*Deepti Mathur, Memorial Sloan Kettering Cancer Center, United States*
*Russell C. Rockne, Beckman Research Institute, City of Hope, United States*

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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *26 February 2021*
***Accepted:*** *22 July 2021*
***Published:*** *17 August 2021*

***Citation:***
*Kareva I and Brown JS (2021) Estrogen as an Essential Resource and the Coexistence of $ER^+$ and $ER^-$ Cancer Cells. Front. Ecol. Evol. 9:673082. doi: 10.3389/fevo.2021.673082*

## INTRODUCTION

Food-webs within ecosystems describe the trophic relationship between species of an ecological community. There can be predators, prey and resources, where different species find themselves consuming those on a lower trophic level, while being consumed by those on a higher one (Rosenzweig, 1971; Oksanen et al., 1981). Predators exploit prey, prey exploit resources, and

resource renewal fuels the food-web. For species on the same trophic level, competition is often indirect. One individual competes with another by consuming and depressing the availability of shared resources. Such ecosystems may have two, three, four, or possibly even more trophic levels (Oksanen and Oksanen, 2000).

A key element of consumer-resource dynamics is the nutritional relationship of resources to the consumer, often showcased by the "beer and pretzel" example of complementary resources. To a consumer, two resources may be perfect substitutes, complementary, hemi-essential or essential (Tilman, 1980). Two nutrients are perfect substitutes if the value of a given diet is a linear, weighted average of the two nutrients in the diet. They are complementary if there are diminishing returns to fitness from consuming more of one of the resources. They are hemi-essential if (i) a non-zero amount of each must be consumed, (ii) there are diminishing returns to consuming more of one, and (iii) consuming more of one resource increases the value of consuming the other (Letnic and Crowther, 2013).

Two resources are essential if some ratio of the two must be consumed to achieve higher reward. That is, increasing consumption of the first resource has no value if consumption of the second resource limits the diet's value, and vice-versa. Such resources conform to Liebig's Law of the Minimum (Liebig, 1876). In the context of agriculture, Justus Freiherr von Liebig (von Liebig, 1840; Liebig, 1876) noted that beyond a certain point adding more of one nutrient, such as nitrogen, did not increase yields as some other nutrient, such as phosphorus, was now the limiting resource. With two resources, at any given time just one or the other resource is limiting unless they conform to a specific ratio in the diet. Essential resources impact the dynamics of both more traditional ecosystems, such as plants or microbes, as well as the dynamics within the ecosystem of the human body. Essential resources may characterize the nutrient or molecular requirements of normal cells, as well as cancer cells within their host.

While normal cells are not free-living single celled organisms, they do rely on consumption of blood-born or tissue-generated nutrients that can serve as fuel, as structural molecules, or as functional molecules (Thompson, 2011). Some of these nutrients can be thought of as *general* resources that are used by all cells in the human body (Palm and Thompson, 2017; Amend et al., 2018). These can include glucose, oxygen, and amino acids. Among these, some are essential, such as the essential amino acids (e.g., lysine) that cannot be synthesized from other amino acids or obtained in another way. Many other molecules can be used as fuel or metabolically transformed into the building blocks for structural and functional purposes (Hosios et al., 2016).

Some nutrients can be described as *specific* resources in that only a subset of cell types or tissues need and use them. For instance, in humans only a subset of cells in the liver and brain can, in general, take up and metabolize fructose; most of our cells cannot (Oppelt et al., 2017). Furthermore, in some cases, the need for a specific resource by a subset of cell types has evolved as an adaption for the whole organism to control the proliferation or metabolic activity of these cell subsets without impacting other cells. Hormones are examples of such specific resources that serve to regulate specific cells within specific tissues; examples of such resources include estrogen for glandular tissue in the breast and testosterone for glandular tissue in the prostate that are necessary for these cells to proliferate. Even as all other nutrients are in ample supply, proliferation of subsets of cells can be controlled by regulating the hormone supply. Hormones, therefore, serve as essential resources relative to the pool of other resources. Yet, as growth factors, they do not provide fuel or material for the cell. Nevertheless, specific cells have evolved to be metabolically wired to require these growth factors as keys (they generally form a dimer with another molecule within the cell (Duffy, 2006; Razandi et al., 2004; Lallous et al., 2013) to initiate metabolic pathways, including the possibility of cell division. They are used up in the process and metabolically broken down. As such, they are a faux resource, whose adaptive value is for the whole organism and not for the individual cell (Tilman, 1982).

Breast cancer cells, at least initially, carry the ancestral trait of requiring estrogen as an essential resource. The ability of cells to recognize and utilize estrogen is mediated through estrogen receptors (ER), which have been an appealing therapeutic target for patients with breast cancer since their discovery over a century ago. Beatson (1896) first observed in 1896 that removing the ovaries can lead to breast cancer remission. Over half a century later, estrogen and its receptors were confirmed as key actors in breast cancer (Jensen et al., 1971), marking the beginning of therapies to interfere with ER signaling to treat the disease.

Once a patient's breast cancer is clinically detectable, cells of the tumor can be classified as $ER^+$ (requiring estrogen) or $ER^-$ (lacking estrogen receptors) by immunohistochemical staining of tumor biopsies. Most primary breast cancers possess both types of cells coexisting within the tumor. Breast cancers are scored pathologically as $ER^+$ or $ER^-$ based on the percentage of cells exhibiting the estrogen receptor. Patients with $ER^+$ breast cancer typically have a more favorable prognosis compared to $ER^-$ patients, with the arsenal of therapeutic interventions expanded to include therapies that interfere with estrogen production or estrogen signaling. Women that score as $ER^-$ have fewer therapy options (Hammond et al., 2010).

Ecologically, within a breast cancer tumor we expect to observe at least three distinct types of communities: all $ER^+$, all $ER^-$ or a community of the two coexisting together. Coexistence seems to be the norm (Jensen et al., 1971; Harvey et al., 1999; Caruana et al., 2020). Here, we want to leverage ecological insights about consumer-resource dynamics and resource subsistence levels to explore the circumstances favoring $ER^+$ vs. $ER^-$ breast cancer. We explore the possibilities for evolutionarily steering cancer cell frequencies through nutrient manipulations. To achieve this goal, we model interactions of cancer cells with a general (glucose) and a specific (estrogen) resource subject to Liebig's Law of the Minimum as a consumer-resource interaction. We consider nutrient uptake rates, resource supply rates, and the proliferation and survival consequences to cancer cells of their nutrient uptake. We identify conditions favoring $ER^+$ or $ER^-$ cancer cells in the tumor microenvironment and discuss strategies that may impact success of hormone-based therapeutic interventions.

## MODEL DESCRIPTION

In our modeling framework, we consider two types of consumers. Consumers using strategy 1, $S_1$, use both specific and general resources ($ER^+$ cells), while consumers using strategy 2, $S_2$, rely only on general resources ($ER^-$ cells). Evidence suggests that in some cases these consumer strategies are heritably distinct and as such are pure strategies that breed true. In other cases, cancer cells can switch between the strategies.

Let the individuals within the population of cancer cells be denoted as $x_\alpha(t)$, where $\alpha$ represents a mixed strategy of using $S_1$ with probability $\alpha$. If $\alpha = 1$, cells only use $S_1$, and if $\alpha = 0$, cells only use $S_2$.

Cells utilizing strategy $S_1$ depend on both the specific resource$R_1(t)$, such as estrogen, and the general resource$R_2(t)$, such as glucose, as essential resources to the cell. Parameters $a_{ij}$ (per time) represent the probability of encountering a given item of the resource, while parameters $b_{ij}$represent the conversion rate of resources into the proliferation daughter cells; we also assume that there exists some intrinsic cell death rate $\delta$. Finally, we can describe the fitness (per capita proliferation rate), $F_1(t)$, of cells using strategy $S_1$ based on Liebig's Law of the Minimum as $F_1(t) = \min(b_{11}a_{11}R_1(t), a_{21}b_{21}R_2(t))$. Cells that use strategy $S_2$ depend only on general resource $R_2(t)$ and grow at a rate $F_2(t) = a_{22}b_{22}R_2(t)$. Together, the change over time in a population with $ER^+$ ($x_{\alpha=0}$) and $ER^-$ ($x_{\alpha=1}$) cancer cells can be described as:

$$\begin{aligned} x'_{\alpha=1} &= x_{\alpha=1}F_1(R_1, R_2) - \delta x_{\alpha=1} \\ x'_{\alpha=0} &= x_{\alpha=0}F_2(R_2) - \delta x_{\alpha=0}. \end{aligned} \quad (1)$$

Next, we assume that resources $R_1(t)$ and $R_2(t)$ have constant inflow rates $R_{01}$ and $R_{02}$, respectively, and are cleared or consumed by normal cells at rates $k_1$ and $k_2$ per unit of the resources $R_1(t)$ and$R_2(t)$, respectively. Resource $R_2(t)$ can be consumed by cells using strategy $S_1$ or strategy $S_2$, while $R_1(t)$ can only be consumed by cells using strategy $S_1$. These assumptions are captured by the following system of equations:

$$\begin{aligned} \frac{dx_{\alpha=1}(t)}{dt} &= x_{\alpha=1}(t)\min(a_{11}b_{11}R_1(t), a_{21}b_{21}R_2(t)) \\ &\quad -\delta x_{\alpha=1}(t) \\ \frac{dx_{\alpha=0}(t)}{dt} &= x_{\alpha=0}(t)a_{22}b_{22}R_2(t) - \delta x_{\alpha=0}(t) \\ N(t) &= x_{\alpha=1}(t) + x_{\alpha=0}(t) \\ \frac{dR_1(t)}{dt} &= R_{01} - k_1R_1(t) - \alpha x_{\alpha=0}(t)\min \\ &\quad \left(a_{11}R_1(t), a_{21}R_2(t)\frac{b_{21}}{b_{11}}\right) \\ \frac{dR_2(t)}{dt} &= R_{02} - k_2R_2(t) - \alpha x_{\alpha=0}(t)a_{21}\min\left(a_{11}R_1(t)\right. \\ &\quad \left.\frac{b_{11}}{b_{21}}, a_{21}R_2(t)\right) - (1-\alpha)x_{\alpha=1}(t)a_{22}R_2(t) \end{aligned} \quad (2)$$

In formulating these dynamics, we assume that cancer cells using strategy $S_1$ do not overconsume either of the essential resources. If resource 1 is limiting, then the cancer cell will consume all encountered items of resource 1 but only some of the encountered items of resource 2. The amount of resource 2 consumed when it is not limiting is just that amount needed to fully utilize resource 1. And vice-versa if resource 2 is limiting.

## EQUILIBRIUM CONDITIONS

In the absence of consumption by the cancer cells, resources will achieve a steady state level at $R'_1 = \frac{R_{01}}{k_1}$ for specific resource $R_1$, and $R'_2 = \frac{R_{02}}{k_2}$ for general resource $R_2$. In consumer-resource theory, these levels are referred to as *resource supply points*. This represents the highest standing crop of resources that is achievable within the tumor.

Each consumer strategy, $S_1$ and $S_2$, will have a subsistence level of resource abundance, $R_1{}^*$ and $R_2{}^*$, below which it will have negative fitness (declining numbers) and above which fitness will be positive (increasing numbers). The subsistence level of resources can be found by setting the fitness of a consumer strategy equal to zero and solving for the associated $R^*$.

When $\alpha = 1$, cells require both resources ($ER^+$ cells), and thus subsistence levels for both resources are $R_1^* = \frac{\delta}{a_{11}b_{11}}$ when $R_2 \geq \frac{\delta}{a_{21}b_{21}}$, or $R_2^* = \frac{\delta}{a_{21}b_{21}}$ when $R_1 \geq \frac{\delta}{a_{11}b_{11}}$. That is, these are the minimal levels of both resources that an $ER^+$ cell requires for survival. In consumer resource theory, the graph of this in the state space of $R_2$ vs. $R_1$ forms an elbow, and it describes the *zero net growth isocline* (ZNGI) for a consumer harvesting essential resources (see **Figure 1**).

Conversely, for cells with $\alpha = 0$, which depend only on the general resource $R_2$, the resource subsistence level is given by $R_2^* = \frac{\delta}{a_{22}b_{22}}$. In the state space of $R_2$ vs. $R_1$, this describes a horizontal line, and it is the zero net growth isocline of strategy $S_2$ (**Figure 1**). Above this line, cells with strategy $S_2$ have positive fitness and below this line their fitness is negative. By the definition of this strategy, the fitness of individuals with $S_2$ is independent of the availability of the specific resource, $R_1$.

In a consumer-resource model there are limits to growth for the consumers. Consumers, intra- and inter-specifically, compete with each other. This competition is indirect via depletion of the standing crop of resources. This means that each species has a carrying capacity determined by the population size that depresses resource availability to its $R^*$. At population sizes above this level, $R$ will be driven below $R^*$ and the consumer's population growth will be negative, and vice-versa for population sizes below this level.

### Role of Tradeoff

For there to be any possibility for the two consumer strategies to coexist, their ZNGIs must intersect at positive values of $R_1$ and $R_2$ as shown in **Figure 1**. The only way for the ZNGIs to not intersect is if the subsistence level of the general resource is lower for $S_2$ than for $S_1$. In this case, cancer cells with strategy $S_2$ will drive the level of the general resource to the point, where consumers using strategy $S_1$ will starve no matter what the abundance of the specific resource, $R_1$. Thus, for $ER^+$ cancer cells

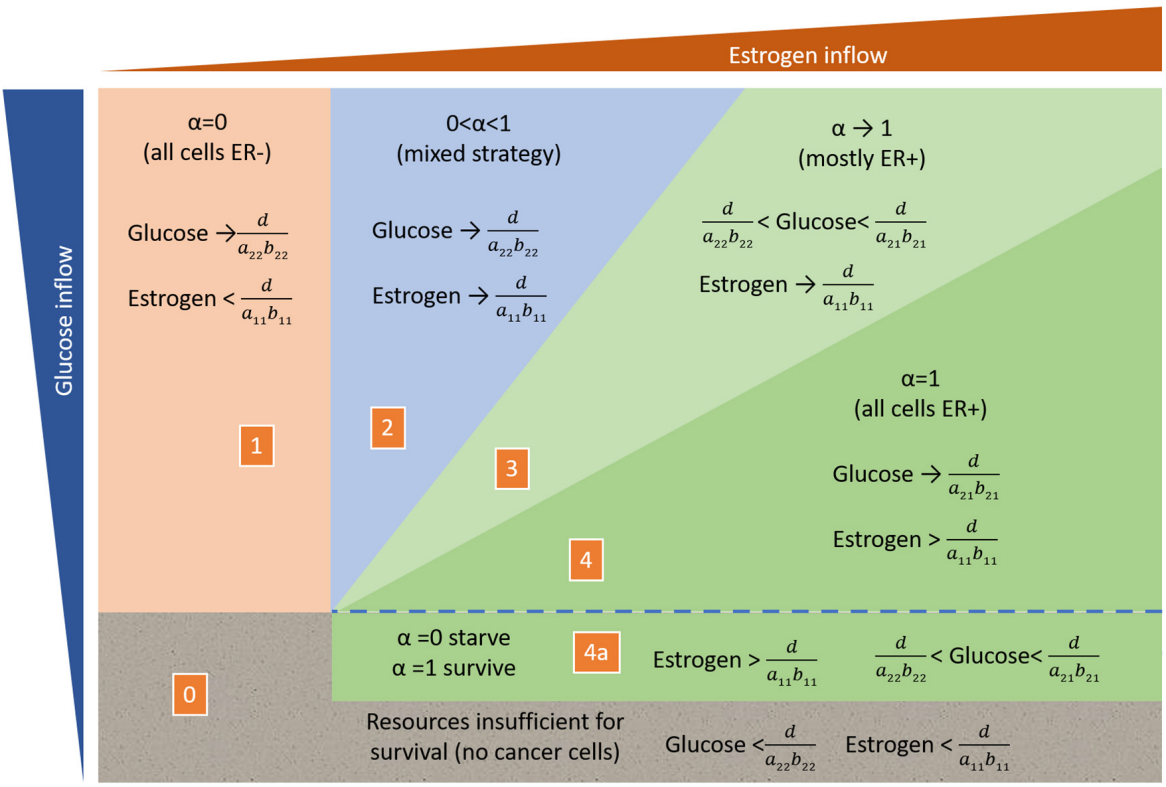


**FIGURE 1 |** Outcome of consumer resource dynamics as influenced by the resource supply point. The five regions show the qualitatively different outcomes described by System (Rosenzweig, 1971).

to persist in the tumor, there must be a tradeoff, such that the $ER^-$ cells free of the specific resource pay the price by having a higher subsistence level on the general resource compared to the $ER^+$ cells: $\frac{\delta}{a_{21}b_{21}} < \frac{\delta}{a_{22}b_{22}}$, which means $a_{21}b_{21} > a_{22}b_{22}$. It is noteworthy that if hormone therapy or time permit $ER^-$ cancer cells to break free of this constraint, then all the cancer cells will be $ER^-$ and unaffected and essentially resistant to all forms of hormonal therapies.

$ER^-$ breast cancers or $ER^+$ cell lines such as MCF-7 that have been selected in the lab to be $ER^-$ exhibit a rewiring of various metabolic pathways (Leung et al., 2010; Nayar et al., 2019). These can include the MAPK/ERK signaling pathways that seem to bypass the estrogen receptor pathway in normal cells of $ER^+$ breast cancer cells (Peng et al., 2017). The rewired metabolic pathways are associated with upregulation of glucose transporters, GLUT1, and increased glycolysis (faster but less efficient use of glucose). Of relevance to our parameter selection, the relative availability of estrogen and glucose alters glucose uptake and metabolism by $ER^+$ MCF-7 cells. In support of the idea that these are essential resources, increased estrogen for MCF-7 cells results in increased glucose uptake and metabolism (Kulkoyluoglu-Cotul et al., 2019). This suggests that the MCF-7 cells had been limited by estrogen, and so had suppressed utilization of glucose. With more estrogen, the amount of glucose that could be usefully utilized was thus increased. For these reasons, we assigned the $ER^-$ a higher encounter rate on glucose than $ER^+$ ($a_{22} > a_{21}$), a lower conversion efficiency ($b_{21} > b_{22}$), and a lower overall product ($a_{21}b_{21} > a_{22}b_{22}$). Beyond satisfying these conditions, the selection of relative magnitudes was arbitrary.

With this tradeoff, $ER^+$ cells have a lower $R^*$ on glucose ( = general resource) than $ER^-$ cells when estrogen has a sufficiently high resource supply. With a surplus of estrogen, $ER^+$ cells can achieve a higher population size than $ER^-$ cells for a given resource supply of glucose. Furthermore, increasing the resource supply of glucose will raise the equilibrium population size of cancer cells. Some evidence supports this prediction. For instance, when grown as mono-cultures in 3-D spheroids, $ER^+$ MCF-7 cells had higher carrying capacities than $ER^-$ MDA-MB-231 cells (Freischel et al., 2020). Whether biopsies of women with $ER^+$ breast cancer exhibit higher densities of cancer cells than those with $ER^-$ breast cancer remains, to our knowledge, an open and interesting question.

## The Role of Resource Supply Points

From here onwards we will assume that this tradeoff exists and that the ZNGIs do cross, as shown in **Figure 1**. The outcomes of the consumer-resource interactions now depend on the resource supply points. Even without competition from consumers using strategy $S_1$, consumer strategy $S_1$ will be absent if the supply points of either of the resources is below subsistence level. Similarly, even in the absence of competition from consumers

using $S_1$, consumer strategy $S_2$ will be absent if the resource supply point for the general resource is below its subsistence level.

*Region 0 of* ***Figure 1***: The cancer cell population cannot sustain itself and will go extinct if the resource supply points are below the subsistence levels of both consumer strategies.

*Region 1 of* ***Figure 1***: Only consumer $ER^-$ cells ($\alpha = 0$) will be present in the cancer cell population if the resource supply point of glucose (general resource) is above $S_2$'s $R^*$ but the resource supply point of estrogen (specific resource) is below the R* of the $ER^+$ cells.

*Region 4a of* ***Figure 1***: Only $ER^+$ cells can be present if the resource supply point is above their subsistence levels of glucose and estrogen, and the resource supply point on glucose is below the $R^*$ of the $ER^-$ strategy.

When the resource supply point, $R$', is above the subsistence $R^*$'s for both the $ER^+$ and $ER^-$ strategies, then there are 3 possible outcomes. In all cases, both strategies could exist in the absence of the other, but the presence of cancer cells using a particular strategy can influence resource depletion in a manner that does not permit both consumer strategies to be present.

*Region 2 of* ***Figure 1***: A mix of $ER^+$ and $ER^-$ cells ($0 < \alpha < 1$) becomes the expected outcome, when the $ER^+$ cells are limited by estrogen and consume so little of the available glucose that they would leave a standing crop of glucose above the R* of the $ER^-$ cells. This outcome becomes likely when the resource supply point exhibits a high ratio of glucose to estrogen. The resulting equilibrium sees the coexistence of both cell types and the depletion of resources to the intersection of the ZNGIs. Namely, the level of estrogen matches the $R^*$ of the $ER^+$ cells, and the level of glucose matches the $R^*$ of the $ER^-$ cells.

*Region 3 of* ***Figure 1***: With a moderate ratio of glucose to estrogen, the $ER^+$ cells will still be limited by estrogen. This means that they leave a level of glucose above their $R^*$; however, if this level of glucose is below the $R^*$ of the $ER^-$ cells, they will slowly and eventually be excluded from the community of cancer cells. In this region, the cancer will tend toward all $ER^+$ ($\alpha = 1$). The standing crop of resources will have estrogen at the $R^*$ of the $ER^+$ cells, and the standing crop of glucose will be above that of the $ER^+$ cells and below that of the $ER^-$ cells.

*Region 4 of* ***Figure 1***: With a low ratio of glucose to estrogen at the resource supply point, the $ER^+$ cells will be glucose limited and not estrogen limited. When this happens, they will drive glucose levels down to their $R^*$ for glucose. Since this is lower than the $R^*$ on glucose for the $ER^-$ cells, the $ER^-$ cells will be outcompeted from this community. One should see a rapid equilibration on a community of just $ER^+$ cancer cells ($\alpha = 1$).

All of the qualitatively different regions shown in **Figure 1** can be solved for analytically from the consumer resource dynamics as summarized in the figure. This analysis allows us to predict the resource-dependent boundaries between different population compositions. Specifically, we can predict resource steady state levels given a fixed population composition with respect to resource consumption strategy. Next, we perform the inverse analysis and predict what composition the population will evolve toward, subject to variations in resource availability and initial population composition.

# INTRODUCING POPULATION HETEROGENEITY WITH RESPECT TO STRATEGY SELECTION

In this section we address the question of how a population that is heterogeneous with respect to resource consumption strategy will evolve over time with respect to both its initial composition and properties of cells and the environment, i.e., with respect to variations in parameters $R_{01}$, $R_{02}$, $a_{ij}$, $b_{ij}$ as defined in **Table 1**. For that, we assume that each individual cell in the population possesses a strategy parameter of $\alpha$ that belongs to the interval [0,1]. With this assumption, the population can consist of individuals that use either the pure strategy (cases analyzed in the previous section), or any mixture of the two pure strategies. There is value in allowing for both possibilities: a mix of two pure strategies vs. a continuum of mixed strategies. In the case of estrogen receptor status in breast cancer, evidence suggests cases in which $ER^+$ and $ER^-$ are heritably distinct and other cases where the trait is phenotypically plastic (Polyak, 2007; Dai et al., 2017; Sahoo et al., 2021).

We can consider the dynamics of any starting distribution of mixed strategies through the application of the Hidden Keystone Variable (HKV) method (Kareva and Karev, 2019); the specific details of transformations necessary to apply the HKV method to this system of equations are given in **Supplementary Appendix**. The final system of equations reads as follows:

$$\frac{dR_1(t)}{dt} = R_{01} - k_1 R_1(t) - E^t[\alpha] N(t) \min\left(a_{11} R_1(t), \frac{a_{21} b_{21} R_2(t)}{b_{11}}\right)$$

$$\frac{dR_2(t)}{dt} = R_{02} - k_2 R_2(t) - E^t[\alpha] N(t) \min\left(\frac{a_{11} b_{11} R_1(t)}{b_{21}}, a_{21} R_2(t)\right) - (1 - E^t[\alpha]) N(t) a_{22} R_2(t) \quad (3)$$

where $E^t[\alpha]$ is the expected value of the strategy parameter that changes over time as the population evolves, and $N(t)$ is the total population size of all the cells. Derivations for expression describing $E^t[\alpha]$ and $N(t)$ are given in **Supplementary Appendix**. Using this transformed system of equations we can now calculate change in population size, expected value and variance of $\alpha$ over time, thus enabling us to track evolution of the population with respect to resource consumption strategy subject to variations in environmental conditions.

# RESULTS

## Model Analysis

We use equations (Oksanen and Oksanen, 2000) to firstly demonstrate the existence of the 4 qualitatively different regimes of coexistence of consumers with the resource, and the resulting final strategy, that are shown in **Figure 1**. The results of our simulations are given in **Figure 2**. We change the inflow rate $R_{01}$ of the specific resource, $R_1$, keeping all other parameter

**TABLE 1 |** Variables and parameters used when there are 2 pure strategies, System (Rosenzweig, 1971), and when there is a distribution of mixed strategies, System (Oksanen and Oksanen, 2000).

| Variables/ parameters | Meaning | Initial conditions/ sample values | Units |
|---|---|---|---|
| $x_\alpha(t)$ | Population density of cells characterized by having a strategy value of $0 \leq \alpha \leq 0$ | $x_\alpha(0) > 0$ | Cells/Vol |
| $N(t)$ | Total population size when there is a distribution of mixed strategies with population sizes denoted by $x_\alpha$; $N(t) = \int_{\mathbb{A}} x_\alpha d\alpha$ | $N(0) = 0.1$ | Cells/Vol |
| $R_1(t)$ | Amount of specific resource (i.e., estrogen) | $R_1(0) = 10$ | Moles/Vol |
| $R_2(t)$ | Amount of general resource (i.e., glucose) | $R_2(t) = 10$ | Moles/Vol |
| $p(t)$ | Auxiliary "keystone" variable necessary for introducing population heterogeneity using the Hidden Keystone Variables (HKV) method (Kareva and Karev, 2019) | $p(0) = 0$ | n/a |
| $q(t)$ | Auxiliary "keystone" variable necessary for introducing population heterogeneity using the HKV method | $q(0) = 0$ | n/a |
| $\alpha$ | Strategy value. If $\alpha = 1$, the cell requires both resources and grows according to Liebig's principle of limiting resources; if $\alpha = 0$, the cell only requires general resource $R_2$. Strategy values $0 < \alpha < 0$ represent mixed strategies | $0 \leq \alpha \leq 1$ | n/a |
| $a_{11}$ | Encounter rate of resource $R_1$ by cells with $\alpha = 1$ | 0.1 | 1/time |
| $b_{11}$ | Rate of conversion of resource $R_1$ into cells with $\alpha = 1$ | 0.1 | $x/R_1$ |
| $a_{21}$ | Encounter rate of resource $R_2$ by cells with $\alpha = 1$ | 0.1 | 1/time |
| $b_{21}$ | Rate of conversion of resource $R_2$ into cells with $\alpha = 1$ | 0.05 | $x/R_2$ |
| $a_{22}$ | Encounter rate of resource $R_2$ by cells with $\alpha = 0$ | 0.25 | 1/time |
| $b_{22}$ | Rate of conversion of resource $R_2$ into cells with $\alpha = 0$ | 0.01 | $x/R_2$ |
| $\delta$ | Natural death rate of cells $x_\alpha(t)$ | 0.01 | 1/time |
| $k_1$ | Natural clearance rate of specific resource $R_1$ | 0.01 | 1/time |
| $k_2$ | Natural clearance rate of general resource $R_2$ | 0.01 | 1/time |
| $R_{01}$ | Inflow rate of specific resource $R_1$ | $k_1 \times R_1(0)$ | $R_1$/time |
| $R_{02}$ | Inflow rate of general resource $R_2$ | $k_2 \times R_2(0)$ | $R_2$/time |
| $\mu$ | Parameter of initial distribution | $-50 < \mu < 50$ | n/a |

values constant as reported in **Table 1**; the initial distribution is assumed to be truncated exponential on the interval [0,1]; other truncated initial distributions can be chosen subject to data availability. We then evaluate changes in total population size N(t) (**Figure 2A**), changes in the standing crop of the specific resource $R_1$ (**Figure 2B**) and the general resource $R_2$ (**Figure 2C**); change in the mean value of the cancer cells' strategy parameter $\alpha$ (**Figure 2D**), changes in the variance of $\alpha$ and change in the population composition over time. Equations used for these calculations are derived in **Supplementary Appendix**.

For the set of parameter values given in **Table 1**, $R_{01} = 5$ corresponds to region 4, where over time the population evolves toward Strategy 1; $\alpha \to 1$, and therefore all the cells in the population require both resources (for the case when $R_1$ represents estrogen, this corresponds to all $ER^+$ cells); the variance (**Figure 2E**) tends to 0 over time, confirming that at steady state, the population is indeed homogeneous with respect to strategy $\alpha$. It is easy to confirm that the equilibrium levels of the specific resource $R_1$ is greater than $\frac{\delta}{a_{11}b_{11}}$, while the equilibrium level of the general resource $R_2$ tends toward $S_1$'s $R^*$ of $\frac{\delta}{a_{21}b_{21}}$, as expected (see **Figure 1**).

Here the model predicts an initially counterintuitive observation: even though the population is composed solely of $ER^+$ cells, there exists a surplus of estrogen (resource $R_1$). However, this makes sense within the framework of Liebig's Law of the Minimum: the general resource $R_2$ is limiting, which results in a surplus of the specific resource $R_1$. This prediction can also have important diagnostic implications, as will be discussed later.

Decreasing the value of $R_{01}$ to 4.5 corresponds to region 3, where final population composition still tends to $\alpha \to 1$ (**Figures 2D,E**). However, the final levels of both resources are different, as can be seen in **Figures 2B,C**. General resource $R_2$ is no longer limiting, and thus its equilibrium levels are higher than in the previous case, while the equilibrium levels of the specific resource $R_1$ are lower. Notably, final population size (**Figure 2A**) is lower in region 3 than in region 4, even though final population composition is nearly identical. In this region, estrogen is the limiting resource for the population of $ER^+$ cells.

Further reducing the value of $R_{01} = 1.1$ corresponds to region 2, which predicts the coexistence of $ER^+$ and $ER^-$ negative cells as a mixed strategy (**Figure 2D**). Notably, this population is heterogeneous at steady state, since its variance over time is non-zero (**Figure 2E**). The change in population composition can also be shown in **Figure 2F**, which plots distribution of cell clones with respect to $\alpha$ over time. As one can clearly see, the population composition changes over time but does not become concentrated at a single value of $\alpha$, as happens for the other cases (not shown). Here, specific resource $R_1$ is limiting for $ER^+$ cells (**Figure 2B**), and thus the level of general resource $R_2$ is at the highest level possible. This level corresponds to the R* of the $ER^-$ cells.

Finally, reducing $R_{01}$ to 0.5, effectively minimizing the level of specific resource $R_1$ below subsistence levels, predictably results in a population that consists entirely of $ER^-$ cells ($\alpha \to 0$). This population has the smallest final population size (**Figure 2A**) and is fully limited by the general resource$R_2$ .

These simulations confirm that the model described by System (Oksanen and Oksanen, 2000) exhibits the outcomes predicted and summarized in **Figure 1**. The simulations show the consumer-resource dynamics toward these outcomes and equilibria. The model tracks changes in the strategy distribution and the final population composition. The simulations verify

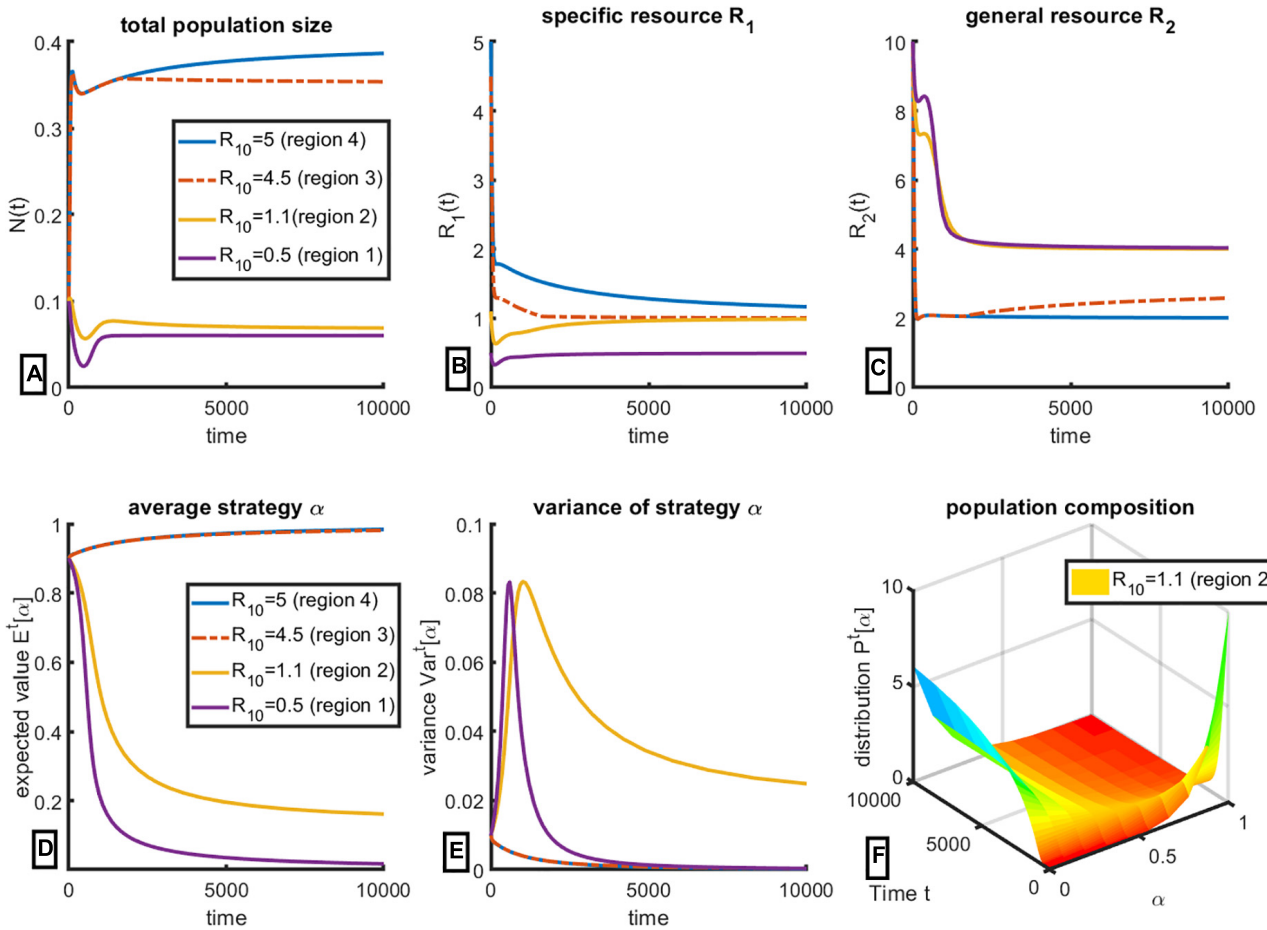


**FIGURE 2 |** Evolution of the population of consumers over time with respect to resource consumption strategy subject to change in resource availability. Initial values of $R_{01}$ were chosen to provide representative plots of the 4 key regions of **Figure 1**. **(A)** Change in total population size N(t); **(B)** Equilibrium level of specific resource $R_1$ (here interpreted as estrogen) and **(C)** equilibrium level of general resource $R_2$, here interpreted as glucose, calculated from System (Oksanen and Oksanen, 2000); **(D)** change in mean strategy $\alpha \in [0, 1]$; **(E)** change in variance of $\alpha$ over time; **(F)** a representative plot of change in population composition changes over time, for $R_{01} = 1.1$, corresponding to region 2 of **Figure 1**. All other parameter values are held constant at values given in **Table 1**.

that changing the ratio of the resource supply points of the two resources results in corresponding changes in the standing crop of the two resources, determining whether the tumor is expected to have all $ER^+$, all $ER^-$ or a mix of both cell types. We additionally demonstrate that populations with mixed equilibrium strategy are heterogeneous at steady state (rather than being composed of a single cell clone). Finally, we note that final population size of cancer cells is largest when $ER^+$ cells dominate the tumor, i.e., cells with largest values of $\alpha$. This last result happens because $ER^+$ dominated tumors occur when the $ER^+$ cells are limited by the general resource and not the specific resource. Since $ER^+$ cells are more efficient on the general resources (lower R*), they can support a larger population size than if it were a tumor composed of $ER^-$. Whether cancers scored as $ER^+$ have a higher overall density of cancer cells than those scored $ER^-$ provides a testable prediction of the model.

Next, we evaluate how composition of the initial population affects the steady state strategy distribution.

## Final Population Composition Is Invariant to Initial Distribution of Cell Clones in the Population

In the previous section we demonstrated that relative levels of specific and general resources affect final population composition. Now we evaluate the impact of initial population composition on final strategy distribution.

For that, we change the value of parameter $\mu$ in Equation (Thompson, 2011) that dictates the initial distribution of mixed strategies in the cancer cell population to see how populations with different initial mean values of $\alpha$ change over time. In the following we hold all other parameters constant at values given in **Table 1** unless indicated otherwise. A representative plot of a population in region 2 of **Figure 1** is given in **Figure 3**.

As one can see, changes in initial population composition do not affect the steady state value of $\alpha$ or the variance of the population; they only affect time necessary to reach the steady state, which is expected. From this we can conclude that within

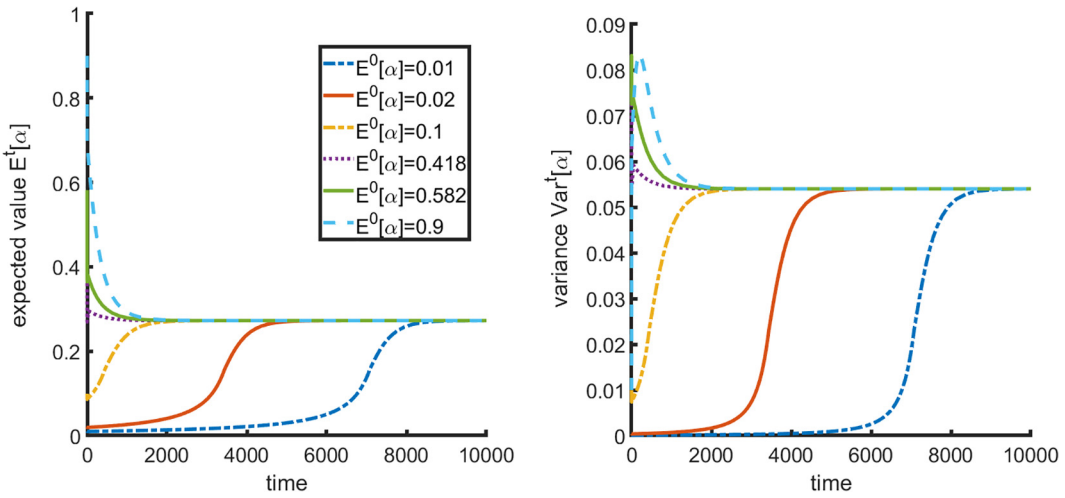


**FIGURE 3 |** Impact of initial distribution on final population composition. **(A)** Final mean strategy α and **(B)** variance are invariant to initial distribution.

the frameworks of the proposed model, it is the relative resource supply points that will drive the evolution of the population over time, and thus it may be possible to steer population composition by manipulating resources.

## Evolution of Population With Respect to Resource Availability

Next, we construct a more integrated picture of the dependence of population composition and resource availability on final strategy at steady state. The data were collected as follows: for various combinations of resource supply rates $R_{01} \in [0.1,\ 20]$ and $R_{20} \in [0.1,\ 10]$, the simulation was run until the population reached a steady state, at which point the corresponding values for total population size (**Figure 4A**), specific resource $R_1$ (**Figure 4B**), general resource $R_2$ (**Figure 4C**), average strategy α (**Figure 4D**), and average variance of α (**Figure 4E**) were noted. Additionally, we plotted the relationship between final population size and final average strategy at steady state in **Figure 4F**, showing clearly that final population size increases as $\alpha \rightarrow 1$.

As expected, final population size is predicted to be largest when both resources are most abundant (**Figure 4A**). The resulting equilibrium abundances of the two resources do not directly depend on the resource supply points but on the resulting community composition. When the resource supply point of the general resource is high and that of the specific resource low, a mixed strategy results. Over this region of coexistence, the specific resource will equilibrate on the subsistence level $R^*$ of consumer strategy 1 (**Figure 4B**), and the general resource will equilibrate on the subsistence $R^*$ of strategy 2 (**Figure 4C**). As changing the ratio of resource supply points shifts the system from mixed strategies to all $S_1$ ($\alpha \rightarrow 1$), the limiting resource switches from the specific to the general resource. Once this happens, the general resource will always equilibrate on $S_1$'s $R^*$ for that resource (**Figure 4C**), and the specific resource at equilibrium will continue to increase (**Figure 4B**) as the ratio of the general to specific resource declines. This happens because a smaller and smaller proportion of the encountered items of the specific resource will actually be consumed by the $S_1$ cancer cells.

In **Figure 4D** we can see that indeed there exists a range of intermediate mixed strategies between regions of evolution toward pure strategies $\alpha \rightarrow 0$ and $\alpha \rightarrow 1$, in correspondence with the theoretically predicted regions described in **Figure 1**. Moreover, **Figure 4E** shows that highest variance, and thus highest degree of population heterogeneity, is observed for populations with intermediate values of α. This may have therapeutic implications, since more heterogeneous populations of cancer cells may indicate a more aggressive cancer in terms of developing metastases or resistance to therapy (Marusyk and Polyak, 2010; Rajput et al., 2017; Marusyk et al., 2020).

# IMPLICATIONS FOR HORMONAL THERAPIES OF BREAST CANCER

The direct impact of nutrient inflow rates on population composition raises the possibility of "evolutionary steering" (Stanková et al., 2019), aimed at promoting a more therapeutically susceptible composition of cancer cell types. Ideally, we would devise strategies to steer the populations toward a point where neither $ER^+$ nor $ER^-$ cells can persist (Region 0 in **Figure 1**). However, this cannot be achieved directly through nutrient manipulation without harming the host, since the general resources (glucose) are required by all cells of the body.

An alternative approach involves steering the population composition toward the $ER^+$ phenotype, which is more susceptible to therapeutic interventions. Such interventions include several endocrine-based therapies, such as tamoxifen, fulvestrant and aromatase inhibitors (AIs). Development of tamoxifen, initially a contraceptive, has been a critical advance in breast cancer treatment (Jordan, 2003; Quirke, 2017). It acts as a selective ER modulator (SERM), interfering with signaling

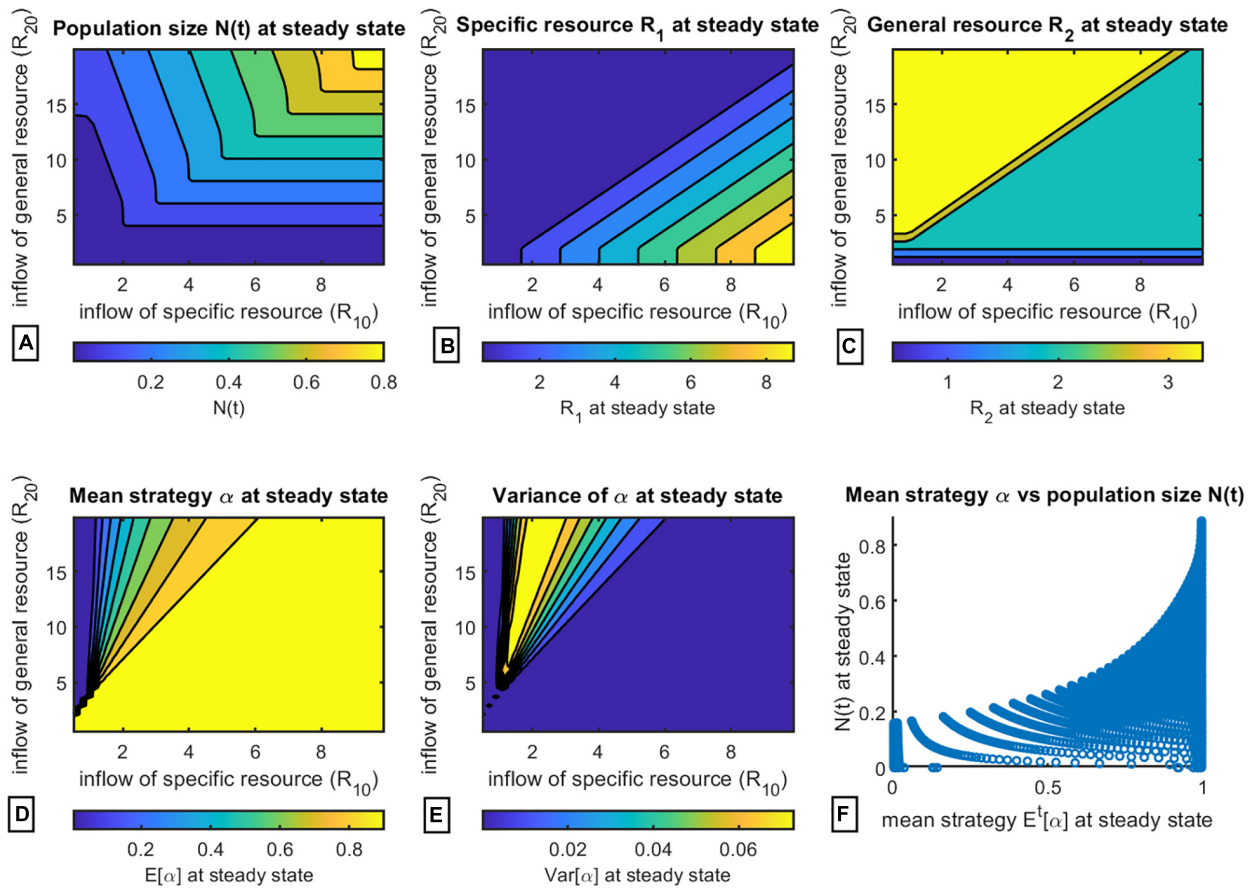


**FIGURE 4 |** Evaluation of population composition and equilibrium values at steady state subject to variation of resource inflow rates. **(A)** population size N(t); **(B)** equilibrium levels of specific resource $R_1$; **(C)** equilibrium levels of general resource $R_2$; **(D)** mean strategy $E^t[\alpha] \in [0, 1]$; **(E)** variance $Var^t[\alpha]$; **(F)** mean strategy $E^t[\alpha]$ vs. population size $N(t)$ at steady state.

between ER and estrogen, although it has been shown to have both antagonist and weak agonist activity. Fulvestrant acts to not only block but also downregulate ER without agonist activity (Osborne et al., 2004). Both are effective in breast cancer, yet both can select for resistant cancer cells (Riggins et al., 2007; Mills et al., 2018), namely those that are $ER^-$ or resistant through other mechanisms. AIs are small molecules that block conversion of precursor compounds into estrogenic molecules (Smith and Dowsett, 2003). AIs, such as anastrozole, letrozole, and exemestane, have proven effective as monotherapies (Mauri et al., 2006) and in combination with tamoxifen (Johnston et al., 2005; Winer et al., 2005; Early Breast Cancer Trialists' Collaborative Group (Ebctcg), 2015).

With a range of options available for estrogen-dependent tumors, it is particularly important to provide therapeutic options to all patients who can benefit. Breast tumors can harbor a combination of $ER^+$ and $ER^-$ cells, but what fraction of $ER^+$ cells within the tumor is high enough to qualify the patient for hormone therapies? This question is not as straightforward as one might believe. One issue concerns inconsistency between testing facilities in how they classify tumors as $ER^+$ or $ER^-$. Typically, one or several sections from a biopsy are stained for ER expression using immunohistochemistry (IHC). Tumors may show a continuum of expression levels among the constituent cancer cells, some cells showing no expression at all ($ER^-$ cells). Generally, all cells exhibiting expression "at any intensity" are reported as positive (Hammond et al., 2010). However, Layfield et al. (2003) showed that there exists considerable variability between ER classifications on the same tissue block when analyzed by different laboratories. Similar discrepancies have been reported by Goldstein et al. (2003) and Nkoy et al. (2010) highlighting differences in laboratory protocols (Ibarra et al., 2010). A nationwide assessment of positivity rates in the Netherlands (Dooijeweert et al., 2019) identified limited variability in a more recent analysis, but absolute variations still existed.

Next, even if there were no inter-laboratory inconsistencies, another question remains: what level of ER expression within the tumor is therapeutically relevant? Two main scoring methods have been used for evaluating the extent of ER positivity: H-score and Allred score. Allred score (Allred et al., 1998) combines the proportion of positive-staining tumor cells and the intensity of staining to give a score between 0 and 8. H-score (Goulding et al., 1995) aims to capture the full range of staining percentages and

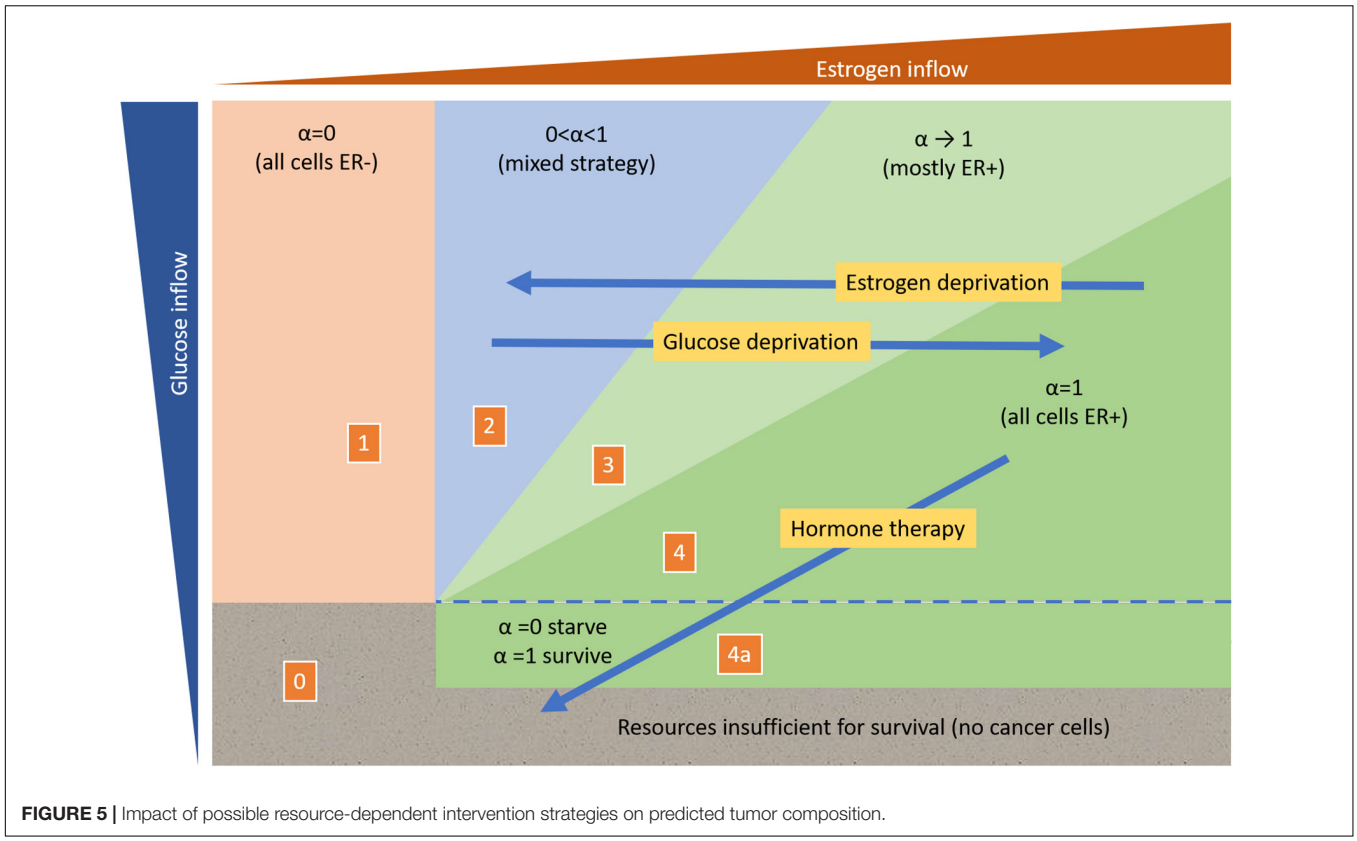


**FIGURE 5 |** Impact of possible resource-dependent intervention strategies on predicted tumor composition.

intensities in tumor samples rather than just the average intensity of the Allred score. H-scores range from 1 to 300. A larger score corresponds to a higher intensity of staining. A score of 1 corresponds to up to 1% $ER^+$ cells among the cancer cells within the tumor.

Some studies however suggest that there are actually few cases of tumors being weakly $ER^+$ vs. entirely $ER^-$. They suggest that ER staining results in a bimodal distribution of tumor types (Collins et al., 2005). The lack of consistency in testing and potential misclassification of weakly $ER^+$ tumors as $ER^+$ tumors prompted Collins et al. (2005) to perform internal IHC analysis of immune-stains of 825 breast cancer samples, estimating proportion of $ER^+$ tumor cells, and grading samples using the Allred score. The authors showed that in 817 cases (99.0%), either all of the cancer cells in the tumor showed an absence of staining (all $ER^-$ cells) or over 70% of the cells were $ER^+$. Thus, 818 cases (99.2%) exhibited Allred scores of either 0 or 7/8. These results prompted the authors to conclude that in most cases, an overwhelming number of breast cancer patients' tumors can be classified as completely $ER^-$ or unambiguously $ER^+$, with only a small fraction of tumors showing very small frequencies of $ER^+$ cells and thus appearing weakly positive. It is noteworthy that Allred scores of 7 or 8 means there are still a sizeable frequency of $ER^-$ cancer cells within the tumor.

The question of whether even weakly positive tumors should be treated with endocrine therapies was addressed in 2010 at the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) meeting. A panel of experts agreed on setting a threshold of 1% $ER^+$ cancer cells for reporting a patient's breast cancer as ER positive (Hammond et al., 2010). With regards to scoring, this corresponds to a minimum Allred score of 3, which can be seen with as few as 1–10% weakly staining cells, and an H-score of over 1 (Table 10 in Hammond et al., 2010).

While the main rationale for the 1% cutoff is to expand access to treatment options to as many patients as possible, it may be too low for statistically significant efficacy. Morgan et al. (2011) showed that low levels of expression, defined as H-score $\leq 50$, resulted in lower overall disease-free survival when treated with only endocrine therapy. Chen et al. (2018) showed that patients with $ER^+$ staining between 1 and 9% gained no significant benefit from endocrine therapy, unlike $ER^+$ tumors with over 10% positive staining. Raghav et al. (2012) also showed that patients with tumors with 1–5% ER positive expression gained no clinical benefit from endocrine therapy.

Even though weakly positive tumors are classified as eligible to receive endocrine therapies, it seems that therapeutic success would be greater if one could increase the proportion of $ER^+$ cells in these tumors prior to administering endocrine therapy. Within the framework proposed here, this may be possible through resource manipulation.

As we have shown above, the proportion of $ER^+$ cells that rely on both resources increases with increased estrogen inflow, or with reduced glucose inflow. Perhaps it might be feasible to externally increase estrogen concentration to favor $ER^+$ cells over $ER^-$ ones without compromising patient health, but as

yet, this has not been explored experimentally. Such a therapy would fall into what has been termed an evolutionary gambit or suckers gambit (Maley et al., 2004; Gatenby and Brown, 2020). On the other hand, glucose deprivation may be able to achieve a similar effect (Barbosa and Martel, 2020), priming the tumor to becoming more receptive to hormone therapy by favoring an increase in the frequency of $ER^+$ cells relative to $ER^-$ ones (**Figure 5**).

If the tumor is primarily composed of $ER^+$ cells, then estrogen deprivation therapies will have initial success but might eventually drive the tumor toward either all $ER^-$ or a mixture of both $ER^+$ and $ER^-$ cells. Adding glucose deprivation or enhancing the resource supply of estrogen relative to other nutrients may set up an evolutionary double-bind (Gatenby et al., 2009), where the resulting resource dynamics force an $ER^+$ tumor that is highly susceptible to hormone therapy (**Figure 5**).

## DISCUSSION

Here we analyzed a consumer-resource model with two resources subject to Liebig's Law of the Minimum to describe evolution of a heterogeneous population of cancer cells as influenced by resource availability. We evaluated the impact of a general resource, such as glucose, and a specific resource, such as estrogen, on the conditions for coexistence by a phenotype that requires both, and a phenotype that only requires the general resource. Our model was intended for breast cancer, where $ER^+$ and $ER^-$ cancer cells are frequently found coexisting within the same patient's tumor. We solved analytically for conditions under which the tumor should have pure or mixed strategies (**Figure 1**). This involved calculating the subsistence levels of resources ($R^*$) for the $ER^+$ and $ER^-$ cancer cell strategies, there zero net growth isoclines (ZNGI), and the effect of the resource supply points of glucose and estrogen on the composition of cancer cell strategies.

We then confirmed theoretical predictions these results by showing that if a heterogeneous population can evolve over time, it will evolve toward the predicted population composition and resource equilibrium levels. We assessed population evolution by changes in the mean and variance of a distribution of mixed strategies, where a given strategy gives the probability of exhibiting the $ER^-$ or $ER^+$ phenotype (**Figure 2**). We showed that in this system, population evolution is invariant to initial distribution of cell clones in the populations, and that over time the final population composition is dictated only by the supply of each resource (**Figure 3**), suggesting that resource manipulation can be used to impact the composition of the population (**Figure 5**).

To test this hypothesis, we varied relative inflow rates for both the general and specific resource and evaluated where the population evolved over time (**Figure 4**). Specifically, in our simulations we allowed the population to evolve to steady state, at which point we evaluated composition of the population (mean strategy and variance of strategies), as well as equilibrium abundances of resources. In addition to confirming predicted levels of both resources at a steady state, the model analysis revealed that the highest variance in the mixed strategies found among the cell lineages occurs for populations that have a mix of $ER^+$ and $ER^-$ cells.

In Lloyd et al. (2014) examined the frequency of $ER^+$ and $ER^-$ cancer cells from the biopsies of 24 patients; all biopsies were obtained from the primary tumor. Six exhibited 100% $ER^-$ cells (corresponding to Region 1 of **Figure 5**), seven had both phenotypes at 5–10% $ER^-$ cells (corresponding to Region 2 of **Figure 5**) and 11 were 100% $ER^+$ cells (corresponding to Regions 3 or 4 of **Figure 5**). The authors found that $ER^-$ tumors exhibit less vasculature. Lack of vasculature may reduce the inflow of both glucose (and other general resources) and estrogen, but the level of estrogen may drop below the subsistence level of the $ER^+$ cells, leaving a higher standing crop of underutilized general resources, thereby favoring $ER^-$ cells (to our knowledge, this prediction remains untested). The authors hypothesize that anti-estrogen therapy (e.g., Tamoxifen) can select for $ER^-$independent cells, while "cyclic introduction of estrogen may improve survival rate by continually altering, rather than unilaterally shifting, toward an $ER^-$ population." The authors suggest that "modulation (and not eradication or extinction of certain population) may prove to be an advantageous treatment strategy," a hypothesis that is supported by the proposed mathematical model.

The proposed model is built on the underlying theory of essential resources and Liebig's Law of the Minimum. For $ER^+$ cells, estrogen may be a hemi-essential resource (it certainly is not a perfectly substitutable one). Had we modeled estrogen as a hemi-essential resource for $ER^+$ cancer cells, our results would remain qualitatively unchanged. The model, however, would lose much of its analytic tractability as fitness would now involve the product of consumption rates of estrogen and the general resource.

Direct evidence for estrogen acting as an essential resource is that $ER^+$ cells cannot survive and proliferate in the absence of estrogen, no matter the abundance of other nutrients (Martin et al., 2003; Comsa et al., 2015). Furthermore, in the absence of sufficient quantities of other nutrients such as glucose, fatty acids and amino acids, cancer cells cannot survive or proliferate regardless of the availability of estrogen. Finally, like other resources, estrogen becomes depleted and used up by the cells rather than continuously being recycled (Gudas et al., 1995).

Indirect evidence suggests that estrogen may act as an essential or hemi-essential resource in line with Liebig's Law. Mathews et al. (2020) quantified the effect of long-term glucose deprivation on various cancer cell lines *in vitro*. Cell lines were stabilized at typical human glucose level of 6 mmol/L, with the intervention group then receiving 3 mmol/L of glucose for 90 days. The authors observed that glucose deprivation had different effect on different cell lines, with MDA-MB-231 cell line ($ER^-$), the highly aggressive triple negative breast cancer cell line, being most sensitive to the metabolic intervention, while the non-tumorigenic epithelial cell line MCF 10A ($ER^+$) was least affected. For the purposes of model validation, more decisive experiments involving $ER^+$ and $ER^-$ cell lines should include varying resource availabilities, and mono- vs. co-culturing to then quantify changes in population composition over time (Freischel et al., 2020). If estrogen and general nutrients function as essential

resources, then nutrient modulation may be an effective strategy for cancer modulation.

The potential benefit on cancer therapy of glucose deprivation through a ketogenic diet has been discussed extensively in the last several years (Klement, 2017; Weber et al., 2018). Khodabakhshi et al. (2020) reported results of a randomized controlled clinical trial, evaluating the effects of ketogenic diet on patients with breast cancer undergoing chemotherapy. In neoadjuvant patients, they found that overall survival increased in the intervention group compared to the control group. In another trial by the same authors (Khodabakhshi et al., 2021) evaluated changes in biomarkers of breast cancer patients undergoing chemotherapy. Patients on a ketogenic diet showed significant decreases in TNF-alpha and insulin levels after 12 weeks of treatment, as well as increase in IL-10. All of these changes are associated with better patient outcomes. Additional experiments are needed to evaluate relative contributions of different mechanisms triggered by glucose deprivation in the presence and absence of estrogen. In addition to manipulating cancer-cell population composition and density, glucose deprivation may also influence immune modulation (Chang et al., 2015; Buck et al., 2017; Klein Geltink et al., 2018) and vasculature (Lloyd et al., 2014).

## Broader Context Within Cancer

The model applies to any cancer that is dependent on specific growth factors including androgen-dependent prostate cancers. Epidermal growth factors (EGF) are typical regulators of many tissue types and they can influence cell proliferation and cell differentiation. Cancers that are wildtype for EGFR (epidermal growth factor receptor) require EGF as an essential resource. Other cancer cells, such as EGFR mutant lung cancer, mutate so that the receptor is permanently turned on, produce their own growth factors or stimulate neighboring normal cells, such as fibroblasts, to produce growth factors for them.

In most of these cases, the growth factor serves as an essential resource necessary for survival and proliferation. The need for these growth factors or hormones derives from the ancestry of the cancer cells. They retain the primitive trait of the normal cells of that tissue type. The need for these growth factors is part of the organism-wide homeostatic control of tissue-specific cell proliferation and activity. Because these hormones and growth factors are not strictly necessary for the survival of an individual cell, there can be strong selection, accelerated by therapy, for a subset of the cancer cells to evolve independence from these. Cancer cell types that are growth factor independent may either replace the others or coexist as a mixed strategy of different phenotypes. We believe our model provides a simple mechanistic explanation for when growth factor independent cancer cell types will either outcompete, coexist with, or be outcompeted by the cancer cell type that requires the growth factor.

## Broader Context Within Consumer-Resource Models

Our model falls well within the class of consumer-resource models proposed and developed by Tilman (1980, 1982). As mentioned in the introduction, in these models, resources can be perfect substitutes, complementary, hemi-essential, or essential. The resources themselves may be co-occurring and encountered at random or distributed in separate patches or habitats (Hunt and Brown, 2018). The nutritional relationship between the resources and their distribution in space strongly influence the potential for the coexistence of different consumers (Vincent et al., 1996).

The analysis of these models has general features described by the resource supply point of the nutrients (inflows), ZNGIs of the consumer species, and the depletion of the resource by the consumers. For models like ours that are non-spatial and achieve a steady-state, coexistence requires that the ZNGIs of the consumers intersect, meaning that there is a tradeoff among consumers between the subsistence levels of the resources. Furthermore, the resource supply points must lie in an intermediate range of the state space of resource abundances but outside of each consumer species' ZNGI. Finally, the consumers must deplete the resources along different trajectories. For a two-resource two-consumer species system, when coexistence is possible, the equilibrium abundance of resources generally lies at the intersection of the two ZNGIs; and the equilibrium population sizes of the two species is what will drive the resource abundances form the supply point to this intersection.

An extensive theoretical and empirical body of literature exists in ecology on consumer-resource dynamics, including essential resources (Abrams, 1987; Fox and Vasseur, 2008). Much of this work is in the context of phytoplankton under chemostat (batch or continuous flow) conditions (Harmand et al., 2017; De Rijcke et al., 2020). The essential resources can be either light and other nutrients, or the nutrients themselves such as nitrogen and phosphorus. Model extensions can include resource pulsing, diffusion gradients within the medium or water column, and large numbers of consumer species and nutrient conditions (Dubinkina et al., 2019; Stojsavljevic et al., 2019).

Tumors can be thought of as rather viscous chemostats, where blood delivers nutrients and removes both residual nutrients and metabolites. In this work, our system was quite simple, with two co-occurring essential resources, applicable to cancers requiring growth hormones or growth factors. More generally, cancers provide a relatively unexplored opportunity to apply and test consumer-resource theory (Palmer et al., 2011; Seynhaeve and Ten Hagen, 2018). Such applications could include ecological "priming" of tumors to be most receptive to therapy, although tracking resource dynamics, cancer cell compositions, and interactions within the tumor is not yet possible. It can at best be inferred from radiographic imaging (MRI, PET scans, CT scans). Jarrett et al. (2020) showed the value of combining MRI and PET scans for inferring cancer cell densities, the distribution of cancer cells with respect to expression of HER2, and mathematically modeling breast-cancer patient responses to neoadjuvant therapies targeting HER2. Their model, like ours, considers the importance of a growth factor (Human epidermal growth factor). Unlike ours, they use a logistic growth model instead of a consumer-resource model; and theirs explicitly considers space using partial differential equations and a diffusion term representing cell dispersal.

Mouse experiments provide greater control and the opportunity to track dynamics more closely, especially using window chamber mouse models (Amend et al., 2018). Culture experiments provide a promising way to compete different cell lines under different nutrient conditions, particularly when grown in 3-D spheroids, where nutrients rather than space become limiting (Freischel et al., 2020). In such experiments the Seahorse Extracellular Flux Analyzers can be used to measure cellular metabolic processes, such as ATP production, glucose consumption, oxygen uptake, lactic acid production and other nutrient fluxes (Cheng et al., 2014; Zhang and Zhang, 2019). Such measure may highlight tradeoffs and differences among cancer cells in uptake and utilization strategies (e.g., glycolytic vs. non-glycolytic cancer cell types) (Persi et al., 2018; Damaghi et al., 2021).

As part of the ecological system of the human body, cancer cells require diverse nutrients, drawing fatty acids, amino acids, trace nutrients, and macromolecules from the blood and interstitial fluid. These nutrients serve as both fuel and as building blocks for structural and functional molecules. Some will be essential, but many will be substitutable or complementary. There is much opportunity to apply consumer-resource dynamics to investigate the ecology, evolution and diversification of cancer cells within and between tumors of a patient, between patients with the same cancer or patients with diverse cancers.

## CONCLUSION

When a patient is diagnosed with breast cancer, it is standard approach to classify the tumor and start tumor treatment as expeditiously as possible, with tumor burden reduction being the goal of each step of the treatment. However, it may be more effective to first prime the tumor following the initial assessment of the frequency of $ER^+$ and $ER^-$ cancer cells. The first step in the treatment cascade could be aimed at modifying the tumor environment to favor $ER^+$ cancer cells. By developing a long-term strategy rather than relying on short-term tumor burden reduction, it may be possible to expand the pool of patients that can maximally benefit from endocrine-based therapy through application of ecological principles to cancer.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

IK conducted MATLAB simulations. Both authors contributed to study design and manuscript preparation.

## FUNDING

This research was partially supported by the grants U54 CA143970, R01 CA077575, and U01CA232382 (to JB).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.673082/full#supplementary-material

## REFERENCES

Abrams, P. A. (1987). The nonlinearity of competitive effects in models of competition for essential resources. *Theor. Popul. Biol.* 32, 50–65. doi: 10.1016/0040-5809(87)90039-6

Allred, D., Harvey, J. M., Berardo, M., and Clark, G. M. (1998). Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod. Pathol.* 11:155.

Amend, S. R., Gatenby, R. A., Pienta, K. J., and Brown, J. S. (2018). Cancer foraging ecology: diet choice, patch use, and habitat selection of cancer cells. *Curr. Pathobiol. Rep.* 6, 209–218. doi: 10.1007/s40139-018-0185-7

Barbosa, A. M., and Martel, F. (2020). Targeting glucose transporters for breast cancer therapy: the effect of natural and synthetic compounds. *Cancers* 12:154. doi: 10.3390/cancers12010154

Beatson, G. T. (1896). Meeting IX.—May 20, 1896: on the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases. *Trans. Med. Chir. Soc. Edinb.* 15:153.

Buck, M. D., Sowell, R. T., Kaech, S. M., and Pearce, E. L. (2017). Metabolic instruction of immunity. *Cell* 169, 570–586. doi: 10.1016/j.cell.2017.04.004

Caruana, D., Wei, W., Martinez-Morilla, S., Rimm, D. L., and Reisenbichler, E. S. (2020). Association between low estrogen receptor positive breast cancer and staining performance. *NPJ Breast Cancer* 6:5.

Chang, C.-H., Qiu, J., O'Sullivan, D., Buck, M. D., Noguchi, T., Curtis, J. D., et al. (2015). Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* 162, 1229–1241. doi: 10.1016/j.cell.2015.08.016

Chen, T., Zhang, N., Moran, M. S., Su, P., Haffty, B. G., and Yang, Q. (2018). Borderline ER-positive primary breast cancer gains no significant survival benefit from endocrine therapy: a systematic review and meta-analysis. *Clin. Breast Cancer* 18, 1–8. doi: 10.1016/j.clbc.2017.06.005

Cheng, G., Zielonka, J., McAllister, D., Tsai, S., Dwinell, M., and Kalyanaraman, B. (2014). Profiling and targeting of cellular bioenergetics: inhibition of pancreatic cancer cell proliferation. *Br. J. Cancer* 111, 85–93. doi: 10.1038/bjc.2014.272

Collins, L. C., Botero, M. L., and Schnitt, S. J. (2005). Bimodal frequency distribution of estrogen receptor immunohistochemical staining results in breast cancer: an analysis of 825 cases. *Am. J. Clin. Pathol.* 123, 16–20. doi: 10.1309/hcf035n9wk40etj0

Comsa, S., Cimpean, A. M., and Raica, M. (2015). The story of MCF-7 breast cancer cell line: 40 years of experience in research. *Anticancer Res.* 35, 3147–3154.

Dai, X., Cheng, H., Bai, Z., and Li, J. (2017). Breast cancer cell line classification and its relevance with breast tumor subtyping. *J. Cancer* 8:3131. doi: 10.7150/jca.18457

Damaghi, M., West, J., Robertson-Tessi, M., Xu, L., Ferrall-Fairbanks, M. C., Stewart, P. A., et al. (2021). The harsh microenvironment in early breast cancer selects for a Warburg phenotype. *Proc. Nat. Acad. Sci.* 118:e2011342118.

De Rijcke, M., Baert, J., Brion, N., Vandegehuchte, M., De Laender, F., and Janssen, C. (2020). Monoculture-based consumer-resource models predict species dominance in mixed batch cultures of dinoflagellates. *Harmful Algae* 99:101921. doi: 10.1016/j.hal.2020.101921

Dooijeweert, C., van, Deckers, I. A., Baas, I. O., Wall, E., van der, et al. (2019). Hormone-and HER2-receptor assessment in 33,046 breast cancer patients: a nationwide comparison of positivity rates between pathology laboratories in the

Netherlands. *Breast Cancer Res. Treat.* 175, 487–497. doi: 10.1007/s10549-019-05180-5

Dubinkina, V., Fridman, Y., Pandey, P. P., and Maslov, S. (2019). Multistability and regime shifts in microbial communities explained by competition for essential nutrients. *Elife* 8:e49720.

Duffy, M. J. (2006). Estrogen receptors: role in breast cancer. *Crit. Rev. Clin. Lab. Sci.* 43, 325–347.

Early Breast Cancer Trialists' Collaborative Group (Ebctcg). (2015). Aromatase inhibitors versus tamoxifen in early. (breast)cancer: patient-level meta-analysis of the randomised trials. *Lancet* 386, 1341–1352. doi: 10.1016/s0140-6736(15)61074-1

Fox, J. W., and Vasseur, D. A. (2008). Character convergence under competition for nutritionally essential resources. *Am. Nat.* 172, 667–680. doi: 10.1086/591689

Freischel, A. R., Damaghi, M., Cunningham, J. J., Ibrahim-Hashim, A., Gillies, R. J., Gatenby, R. A., et al. (2020). Frequency-dependent interactions determine outcome of competition between two breast cancer cell lines. *Sci. Rep.* 11:4908.

Gatenby, R. A., and Brown, J. S. (2020). The evolution and ecology of resistance in cancer therapy. *Cold Spring Harbor Perspect. Med.* 10:a040972. doi: 10.1101/cshperspect.a040972

Gatenby, R. A., Brown, J., and Vincent, T. (2009). Lessons from applied ecology: cancer control using an evolutionary double bind. *Cancer Res.* 69, 7499–7502. doi: 10.1158/0008-5472.can-09-1354

Goldstein, N. S., Ferkowicz, M., Odish, E., Mani, A., and Hastah, F. (2003). Minimum formalin fixation time for consistent estrogen receptor immunohistochemical staining of invasive breast carcinoma. *Am. J. Clin. Pathol.* 120, 86–92. doi: 10.1309/qphdrb00qxgmuq9n

Goulding, H., Pinder, S., Cannon, P., Pearson, D., Nicholson, R., Snead, D., et al. (1995). A new immunohistochemical antibody for the assessment of estrogen receptor status on routine formalin-fixed tissue samples. *Hum. Pathol.* 26, 291–294. doi: 10.1016/0046-8177(95)90060-8

Gudas, J. M., Nguyen, H., Li, T., and Cowan, K. H. (1995). Hormone-dependent regulation of BRCA1 in human breast cancer cells. *Cancer Res.* 55, 4561–4565.

Hammond, M. E. H., Hayes, D. F., Dowsett, M., Allred, D. C., Hagerty, K. L., Badve, S., et al. (2010). American society of clinical oncology/college of american pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). *Arch. Pathol. Lab. Med.* 134, e48–e72.

Harmand, J., Lobry, C., Rapaport, A., and Sari, T. (2017). *The Chemostat: Mathematical Theory of Microorganism Cultures*. Hoboken, NJ: John Wiley & Sons.

Harvey, J. M., Clark, G. M., Osborne, C. K., and Allred, D. C. (1999). Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J. Clin. Oncol.* 17, 1474–1481. doi: 10.1200/jco.1999.17.5.1474

Hosios, A. M., Hecht, V. C., Danai, L. V., Johnson, M. O., Rathmell, J. C., Steinhauser, M. L., et al. (2016). Amino acids rather than glucose account for the majority of cell mass in proliferating mammalian cells. *Dev. Cell* 36, 540–549. doi: 10.1016/j.devcel.2016.02.012

Hunt, V. M., and Brown, J. S. (2018). Coexistence and displacement in consumer-resource systems with local and shared resources. *Theor. Ecol.* 11, 83–93. doi: 10.1007/s12080-017-0350-3

Ibarra, J. A., Rogers, L. W., Kyshtoobayeva, A., and Bloom, K. (2010). Fixation time does not affect the expression of estrogen receptor. *Am. J. Clin. Pathol.* 133, 747–755. doi: 10.1309/ajcppiuhs4gvar0i

Jarrett, A. M., Hormuth, D. A., Adhikarla, V., Sahoo, P., Abler, D., Tumyan, L., et al. (2020). Towards integration of 64 Cu-DOTA-trastuzumab PET-CT and MRI with mathematical modeling to predict response to neoadjuvant therapy in HER2+ breast cancer. *Sci. Rep.* 10:20518.

Jensen, E. V., Smith, K., and Kyser, K. (1971). Estrogen Receptors and Breast Cancer Response to. Prediction of response in cancer therapy. *Natl. Cancer Inst. Mongor.* 34, 55–70

Johnston, S. R., Martin, L.-A., Head, J., Smith, I., and Dowsett, M. (2005). Aromatase inhibitors: combinations with fulvestrant or signal transduction inhibitors as a strategy to overcome endocrine resistance. *J. Steroid Biochem. Mol. Biol.* 95, 173–181. doi: 10.1016/j.jsbmb.2005.04.004

Jordan, V. C. (2003). Tamoxifen: a most unlikely pioneering medicine. *Nat. Rev. Drug Discov.* 2, 205–213. doi: 10.1038/nrd1031

Kareva, I., and Karev, G. (2019). *Modeling Evolution of Heterogeneous Populations. Theory and Applications*. Amsterdam: Elsevier.

Khodabakhshi, A., Akbari, M. E., Mirzaei, H. R., Mehrad-Majd, H., Kalamian, M., and Davoodi, S. H. (2020). Feasibility, safety, and beneficial effects of MCT-based ketogenic diet for breast cancer treatment: a randomized controlled trial study. *Nutr. Cancer* 72, 627–634. doi: 10.1080/01635581.2019.1650942

Khodabakhshi, A., Akbari, M. E., Mirzaei, H. R., Seyfried, T. N., Kalamian, M., and Davoodi, S. H. (2021). Effects of Ketogenic metabolic therapy on patients with breast cancer: a randomized controlled clinical trial. *Clin. Nutr.* 40, 751–758. doi: 10.1016/j.clnu.2020.06.028

Klein Geltink, R. I., Kyle, R. L., and Pearce, E. L. (2018). Unraveling the complex interplay between T cell metabolism and function. *Annu. Rev. Immunol.* 36, 461–488. doi: 10.1146/annurev-immunol-042617-053019

Klement, R. J. (2017). Beneficial effects of ketogenic diets for cancer patients: a realist review with focus on evidence and confirmation. *Med. Oncol.* 34, 1–15. doi: 10.1080/01635581.2020.1836243

Kulkoyluoglu-Cotul, E., Arca, A., and Madak-Erdogan, Z. (2019). Crosstalk between estrogen signaling and breast cancer metabolism. *Trends Endocrinol. Metab.* 30, 25–38. doi: 10.1016/j.tem.2018.10.006

Lallous, N., Dalal, K., Cherkasov, A., and Rennie, P. S. (2013). Targeting alternative sites on the androgen receptor to treat castration-resistant prostate cancer. *Int. J. Mol. Sci.* 14, 12496–12519. doi: 10.3390/ijms140612496

Layfield, L. J., Goldstein, N., Perkinson, K. R., and Proia, A. D. (2003). Interlaboratory variation in results from immunohistochemical assessment of estrogen receptor status. *Breast J.* 9, 257–259. doi: 10.1046/j.1524-4741.2003.09325.x

Letnic, M., and Crowther, M. S. (2013). Patterns in the abundance of kangaroo populations in arid Australia are consistent with the exploitation ecosystems hypothesis. *Oikos* 122, 761–769. doi: 10.1111/j.1600-0706.2012.20425.x

Leung, E., Kannan, N., Krissansen, G. W., Findlay, M. P., and Baguley, B. C. (2010). MCF-7 breast cancer cells selected for tamoxifen resistance acquire new phenotypes differing in DNA content, phospho-HER2 and PAX2 expression, and rapamycin sensitivity. *Cancer Biol. Ther.* 9, 717–724. doi: 10.4161/cbt.9.9.11432

Liebig, J. (1876). "Animal chemistry: or organic chemistry in its application to physiology and pathology," in *A Facsimile of the Cambridge Edition, 1842, With a New Introduction by Frederic L. Holmes*, eds W. Gregory and J. W. Webster (New York, NY: Johnson Reprint Corporation).

Lloyd, M. C., Alfarouk, K. O., Verduzco, D., Bui, M. M., Gillies, R. J., Ibrahim, M. E., et al. (2014). Vascular measurements correlate with estrogen receptor status. *BMC Cancer* 14:279.

Maley, C. C., Reid, B. J., and Forrest, S. (2004). Cancer prevention strategies that address the evolutionary dynamics of neoplastic cells: simulating benign cell boosters and selection for chemosensitivity. *Cancer Epidemiol. Prevention Biomark.* 13, 1375–1384.

Martin, L.-A., Farmer, I., Johnston, S. R., Ali, S., Marshall, C., and Dowsett, M. (2003). Enhanced estrogen receptor (ER) α, ERBB2, and MAPK signal transduction pathways operate during the adaptation of MCF-7 cells to long term estrogen deprivation. *J. Biol. Chem.* 278, 30458–30468. doi: 10.1074/jbc.m305226200

Marusyk, A., and Polyak, K. (2010). Tumor heterogeneity: causes and consequences. *Biochim. Biophys. Acta* 1805, 105–117. doi: 10.1016/j.bbcan.2009.11.002

Marusyk, A., Janiszewska, M., and Polyak, K. (2020). Intratumor heterogeneity: the rosetta stone of therapy resistance. *Cancer Cell* 37, 471–484. doi: 10.1016/j.ccell.2020.03.007

Mathews, E. H., Visagie, M. H., Meyer, A. A., Joubert, A. M., and Mathews, G. E. (2020). In vitro quantification: long-term effect of glucose deprivation on various cancer cell lines. *Nutrition* 74:110748. doi: 10.1016/j.nut.2020.110748

Mauri, D., Pavlidis, N., Polyzos, N. P., and Ioannidis, J. P. (2006). Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis. *J. Natl. Cancer Inst.* 98, 1285–1291. doi: 10.1093/jnci/djj357

Mills, J. N., Rutkovsky, A. C., and Giordano, A. (2018). Mechanisms of resistance in estrogen receptor positive breast cancer: overcoming resistance to tamoxifen/aromatase inhibitors. *Curr. Opin. Pharmacol.* 41, 59–65. doi: 10.1016/j.coph.2018.04.009

Morgan, D. A., Refalo, N. A., and Cheung, K. L. (2011). Strength of ER-positivity in relation to survival in ER-positive breast cancer treated by adjuvant tamoxifen as sole systemic therapy. *Breast* 20, 215–219. doi: 10.1016/j.breast.2010.11.004

Nayar, U., Cohen, O., Kapstad, C., Cuoco, M. S., Waks, A. G., Wander, S. A., et al. (2019). Acquired HER2 mutations in ER+ metastatic breast cancer confer resistance to estrogen receptor–directed therapies. *Nat. Genet.* 51, 207–216. doi: 10.1038/s41588-018-0287-5

Nkoy, F. L., Hammond, M. E. H., Rees, W., Belnap, T., Rowley, B., Catmull, S., et al. (2010). Variable specimen handling affects hormone receptor test results in women with breast cancer: a large multihospital retrospective study. *Arch. Pathol. Lab. Med.* 134, 606–612. doi: 10.5858/134.4.606

Oksanen, L., and Oksanen, T. (2000). The logic and realism of the hypothesis of exploitation ecosystems. *Am. Nat.* 155, 703–723. doi: 10.2307/3079095

Oksanen, L., Fretwell, S. D., Arruda, J., and Niemela, P. (1981). Exploitation ecosystems in gradients of primary productivity. *Am. Nat.* 118, 240–261. doi: 10.1086/283817

Oppelt, S. A., Zhang, W., and Tolan, D. R. (2017). Specific regions of the brain are capable of fructose metabolism. *Brain Res.* 1657, 312–322. doi: 10.1016/j.brainres.2016.12.022

Osborne, C., Wakeling, A., and Nicholson, R. (2004). Fulvestrant: an oestrogen receptor antagonist with a novel mechanism of action. *Br. J. Cancer* 90, S2–S6.

Palm, W., and Thompson, C. B. (2017). Nutrient acquisition strategies of mammalian cells. *Nature* 546, 234–242. doi: 10.1038/nature22379

Palmer, G. M., Fontanella, A. N., Shan, S., Hanna, G., Zhang, G., Fraser, C. L., et al. (2011). In vivo optical molecular imaging and analysis in mice using dorsal window chamber models applied to hypoxia, vasculature and fluorescent reporters. *Nat. Protoc.* 6, 1355–1366. doi: 10.1038/nprot.2011.349

Peng, W., Huang, J., Yang, L., Gong, A., and Mo, Y.-Y. (2017). Linc-RoR promotes MAPK/ERK signaling and confers estrogen-independent growth of breast cancer. *Mol. Cancer* 16:161.

Persi, E., Duran-Frigola, M., Damaghi, M., Roush, W. R., Aloy, P., Cleveland, J. L., et al. (2018). Systems analysis of intracellular pH vulnerabilities for cancer therapy. *Nat. Commun.* 9:2997.

Polyak, K. (2007). Breast cancer: origins and evolution. The Journal of clinical investigation. *Am. Soc. Clin. Investig* 117, 3155–3163. doi: 10.1172/jci33295

Quirke, V. M. (2017). Tamoxifen from failed contraceptive pill to best-selling breast cancer medicine: a case-study in pharmaceutical innovation. *Front. Pharmacol.* 8:620.

Raghav, K. P., Hernandez-Aya, L. F., Lei, X., Chavez-MacGregor, M., Meric-Bernstam, F., Buchholz, T. A., et al. (2012). Impact of low estrogen/progesterone receptor expression on survival outcomes in breast cancers previously classified as triple negative breast cancers. *Cancer* 118, 1498–1506. doi: 10.1002/cncr.26431

Rajput, A., Bocklage, T., Greenbaum, A., Lee, J.-H., and Ness, S. A. (2017). Mutant-allele tumor heterogeneity scores correlate with risk of metastases in colon cancer. *Clin. Colorectal Cancer* 16, e165–e170.

Razandi, M., Pedram, A., Merchenthaler, I., Greene, G. L., and Levin, E. R. (2004). Plasma membrane estrogen receptors exist and functions as dimers. *Mol. Endocrinol.* 18, 2854–2865. doi: 10.1210/me.2004-0115

Riggins, R. B., Schrecengost, R. S., Guerrero, M. S., and Bouton, A. H. (2007). Pathways to tamoxifen resistance. *Cancer Lett.* 256, 1–24. doi: 10.1016/j.canlet.2007.03.016

Rosenzweig, M. L. (1971). Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. *Science* 171, 385–387. doi: 10.1126/science.171.3969.385

Sahoo, S., Mishra, A., Kaur, H., Hari, K., Muralidharan, S., Mandal, S., et al. (2021). A mechanistic model captures the emergence and implications of non-genetic heterogeneity and reversible drug resistance in ER+ breast cancer cells. *bioRxiv [preprint]* doi: 10.1101/2021.03.14.435359

Seynhaeve, A. L., and Ten Hagen, T. L. (2018). Intravital microscopy of tumor-associated vasculature using advanced dorsal skinfold window chambers on transgenic fluorescent mice. *J. Vis. Exp.* 131:55115.

Smith, I. E., and Dowsett, M. (2003). Aromatase inhibitors in breast cancer. New England journal of medicine. *Mass Med. Soc.* 348, 2431–2442. doi: 10.1056/nejmra023246

Stanková, K., Brown, J. S., Dalton, W. S., and Gatenby, R. A. (2019). Optimizing cancer treatment using game theory: a review. *JAMA Oncol.* 5, 96–103. doi: 10.1001/jamaoncol.2018.3395

Stojsavljevic, T., Pinter, G., Lauko, I., and Myers, N. (2019). Parameter identification and sensitivity analysis for a phytoplankton competition model. *Q. Appl. Mathem.* 77, 1–18. doi: 10.1090/qam/1514

Thompson, C. (2011). *Rethinking the Regulation of Cellular Metabolism. Cold Spring Harbor Symposia on Quantitative Biology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Tilman, D. (1980). Resources: a graphical-mechanistic approach to competition and predation. *Am. Nat.* 116, 362–393. doi: 10.1086/283633

Tilman, D. (1982). *Resource Competition and Community Structure*. Princeton, NJ: Princeton University Press.

Vincent, T., Scheel, D., Brown, J., and Vincent, T. (1996). Trade-offs and coexistence in consumer-resource models: it all depends on what and where you eat. *Am. Nat.* 148, 1038–1058. doi: 10.1086/285970

von Liebig, J. F. (1840). *Die organische Chemie in Ihrer Anwendung auf Agricultur und Physiologie*. Cambridge: Cambridge University Press.

Weber, D. D., Aminazdeh-Gohari, S., and Kofler, B. (2018). Ketogenic diet in cancer therapy. *Aging* 10, 164–165.

Winer, E. P., Hudis, C., Burstein, H. J., Wolff, A. C., Pritchard, K. I., Ingle, J. N., et al. (2005). American society of clinical oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for postmenopausal women with hormone receptor–positive breast cancer: status report 2004. *J. Clin. Oncol.* 23, 619–629. doi: 10.1200/jco.2005.09.121

Zhang, J., and Zhang, Q. (2019). Using seahorse machine to measure OCR and ECAR in cancer cells. *Cancer Metabolism* 1928, 353–363. doi: 10.1007/978-1-4939-9027-6_18

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling Editor declared a past co-authorship with one of the authors IK.

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frontiers in Ecology and Evolution

REVIEW
published: 22 September 2021
doi: 10.3389/fevo.2021.681100

# The Genomic Processes of Biological Invasions: From Invasive Species to Cancer Metastases and Back Again

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The concept of invasion is useful across a broad range of contexts, spanning from the fine scale landscape of cancer tumors up to the broader landscape of ecosystems. Invasion biology provides extraordinary opportunities for studying the mechanistic basis of contemporary evolution at the molecular level. Although the field of invasion genetics was established in ecology and evolution more than 50 years ago, there is still a limited understanding of how genomic level processes translate into invasive phenotypes across different taxa in response to complex environmental conditions. This is largely because the study of most invasive species is limited by information about complex genome level processes. We lack good reference genomes for most species. Rigorous studies to examine genomic processes are generally too costly. On the contrary, cancer studies are fortified with extensive resources for studying genome level dynamics and the interactions among genetic and non-genetic mechanisms. Extensive analysis of primary tumors and metastatic samples have revealed the importance of several genomic mechanisms including higher mutation rates, specific types of mutations, aneuploidy or whole genome doubling and non-genetic effects. Metastatic sites can be directly compared to primary tumor cell counterparts. At the same time, clonal dynamics shape the genomics and evolution of metastatic cancers. Clonal diversity varies by cancer type, and the tumors' donor and recipient tissues. Still, the cancer research community has been unable to identify any common events that provide a universal predictor of "metastatic potential" which parallels findings in evolutionary ecology. Instead, invasion in cancer studies depends strongly on context, including order of events and clonal composition. The detailed studies of the behavior of a variety of human cancers promises to inform our understanding of genome level dynamics in the diversity of invasive species and provide novel insights for management.

**Keywords: clonal diversity, epigenomics and epigenetics, genomics, metastasis, non-genetic inheritance, invasion biology, invasive species**

OPEN ACCESS

***Edited by:***
*Sarah R. Amend, Johns Hopkins Medicine, United States*

***Reviewed by:***
*Aurora Mihaela Nedelcu, University of New Brunswick Fredericton, Canada*
*Javad Noorbakhsh, Broad Institute, United States*

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***Specialty section:***
*This article was submitted to Population, Community, and Ecosystem Dynamics, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *15 March 2021*
***Accepted:*** *08 July 2021*
***Published:*** *22 September 2021*

***Citation:***
*Neinavaie F, Ibrahim-Hashim A, Kramer AM, Brown JS and Richards CL (2021) The Genomic Processes of Biological Invasions: From Invasive Species to Cancer Metastases and Back Again. Front. Ecol. Evol. 9:681100. doi: 10.3389/fevo.2021.681100*

## INTRODUCTION

The concept of invasion is provocative across many levels of biology. In the context of biodiversity and ecology, microbial, plant, and animal species invade non-native ecosystems imposing ecological and economic problems and challenges on a global scale (Pimentel et al., 2000; Pyšek and Richardson, 2010; Simberloff et al., 2013; Strong and Ayres, 2013; van Kleunen et al., 2018;

Bartz and Kowarik, 2019; Cuthbertab et al., 2021). In cancers, a primary tumor in one tissue can give rise to lineages that disperse to a wide variety of novel environments in other tissues of the host (Turajlic et al., 2018; Capp and Thomas, 2020), imposing a potentially lethal cost on the host (Pienta et al., 2020a; Dujon et al., 2021). Evolutionary processes are inherent to invasions as biological entities are exposed to environmental conditions that may vary from their original environments. The invasive species may experience population bottlenecks, and be subject to genetic drift (Bock et al., 2015; Sottoriva et al., 2017; Zahir et al., 2020). In studies that span the diversity of biological taxa and known human cancers, comparison of source and invasive populations of species and cancer cells has shed light on how rapid evolution can occur (Lee, 2002; Bossdorf et al., 2005; Prentis et al., 2008; Turajlic et al., 2018; Alexandrov et al., 2020; Gerstung et al., 2020).

The field of invasion genetics was established in ecology and evolution more than 50 years ago to understand the genetic mechanisms underlying invasion in natural systems (Baker and Stebbins, 1965). But even by 2002, evolutionary genetics was considered "relatively unexplored" in most invasive species (Lee, 2002). Despite some level of success in the last two decades, we have only a limited understanding of how genomic processes translate into phenotypic diversity across different taxa in response to complex environmental conditions (Bock et al., 2015; van Kleunen et al., 2018; Mounger et al., 2021a). Several studies have concluded that while population bottlenecks and genetic drift typically have a negative effect on invasion success, adaptive responses by invasive species are not limited by reduced genetic variation (Bock et al., 2015; Dlugosch et al., 2015; Estoup et al., 2016; Colautti et al., 2017). However, the study of most invasive species is constrained by a lack of information about complex genome level processes. We lack good reference genomes for most species. Genomic approaches are expensive, and typically studies have focused only on DNA sequences as the mechanism of inheritance (Bock et al., 2015; Richards and Pigliucci, 2020; Mounger et al., 2021a). Further, information about the genetic make-up of source populations is often limited, and what genetic changes occur during the "**lag time**" (see glossary in **Table 1** for bolded terms) between introduction and invasion is virtually unexplored (Bock et al., 2015; van Kleunen et al., 2018).

There are several ways to consider how cancer can be seen as a process of invasion. This could include the initiation of cancer as a cell lineage goes from being part of the whole organism program to becoming its own self-defined fitness function and unit of selection (Gatenby and Brown, 2017). Furthermore, once initiated, the expanding population of cancer cells evolves rapidly, and invades adjacent unoccupied tissue. Finally, some cells may metastasize to other regions of the same tissue or to novel organs other than that of the primary tumor. Here, we are interested in parallels between cancer and biological invasions with respect to an extant and expanding population of cancer cells as opposed to cancer initiation itself (**Figure 1**). In this context, metastases are described quite similarly to biological invasions in the cancer literature. There are also comparable outstanding questions in both fields of inquiry (**Table 2**). During the process of **metastasis**, cancer cells leave the primary tumor and establish new tumors either in the same or different tissue (Nowell, 1976). Understanding this process is critical considering that metastasis is linked to the majority of cancer-related deaths (Lambert et al., 2017; Birkbak and McGranahan, 2020). Besides creating lethal burdens or organ failures, the metastatic disease eventually evolves resistance to all known therapies (Pienta et al., 2020a).

The process of invasion in diverse cancers provides unique opportunities for studying contemporary evolution since cancer studies are fortified with extraordinary resources for studying genome level dynamics and the interactions among genetic and non-genetic mechanisms (Turajlic et al., 2018; Gerstung et al., 2020). Cancer studies have shown that compared to primary cancer cell counterparts, metastatic samples can have higher mutation rates, multiple types of mutations, and aneuploidy or whole genome doubling with attendant non-genetic effects (Patel and Vanharanta, 2017; Sansregret and Swanton, 2017; Turajlic et al., 2018; Pienta et al., 2020b, 2021; Patel et al., 2021). This suggests that these genome level processes can be important in the invasion process as has also been implicated in the evolutionary ecology of invasive species (Bock et al., 2015; Mounger et al., 2021a). At the same time, bottleneck events shape the genomics and evolution of metastatic cancers (Loeb et al., 2003; Szczurek et al., 2020; Patel et al., 2021), and clonal diversity varies by cancer type and the recipient tissues of the metastases (Turajlic et al., 2018). All the progress in cancer genomics notwithstanding, the cancer research community has been unable to identify any common events that provide a universal predictor

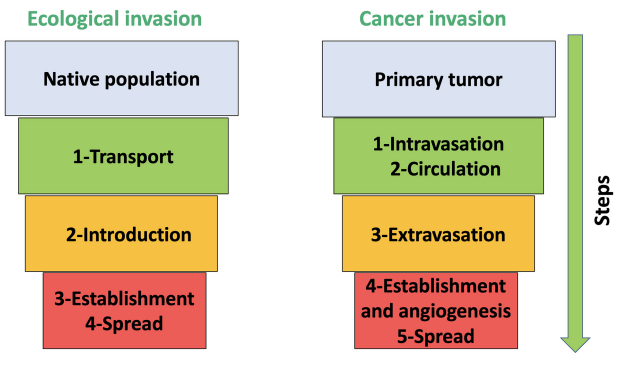



**FIGURE 1 |** A schematic figure that compares the steps of biological invasion in invasive species (adopted from Theoharides and Dukes, 2007) and cancers (adopted from Gatenby et al., 2009). Native population and primary tumor (in light blue) refer to the source of ecological invasion and cancer invasion, respectively. The first step (green) for invasive species is transport in ecological invasion, which can be natural or by human-assisted dispersal. This step resembles two steps in cancer invasion: (1) intravasation and (2) circulation, where a non-random subset of cancer cells first enters the blood or lymphatic vessels and then travels to a distant organ(s). The following step in the ecological invasion (2) introduction (in orange) closely mimics step three in cancer invasion: extravasation. In invasive species and cancer cells, this step refers to the introduction to the new site. The final steps in the ecological invasion, (3) establishment and (4) spread, resembles in cancer (4) establishment and extension into the adjacent tissue, and (5) colony formation, angiogenesis, proliferation, and spread.

**TABLE 1 |** Glossary of relevant terms used to describe biological invasion in invasive species and cancers.

| |
|---|
| **Angiogenesis –** A term that refers to accessing and establishing new vasculature (for example, within a tumor). |
| **Basement membrane –** The thin membrane that separates the epithelium (for example, cancer cells) from the underlying tissues (for example, blood vessels). |
| **Biotic resistance –** An ecological term that refers to resistance to natural enemies like herbivores or pathogens. |
| **Circulating tumor cells (CTC) –** A cancer term that refers to the subset of cancer cells that can be detected in the blood of a patient diagnosed with a primary solid tumor or metastasis. |
| **Dispersal corridors –** An ecological term that refers to a path that links two or more favorable habitats. |
| **Disseminated tumor cells (DTC) –** A cancer term that refers to a subset of cancer cells that can be detected in the bone marrow or other organs that dispersed from the primary tumor or a secondary tumor. |
| **Drivers –** A term that refers to specific mutations that can have large effects (for example, leading to cancer development). |
| **Epithelial to mesenchymal transition –** A term that refers to a dynamic change in cells from epithelial phenotype to mesenchymal phenotype. |
| **Extravasation –** A term that refers to the invasion process of cancer cells exiting the blood vessels in the distal organ for colonization. |
| **Evolution of increased competitive ability –** An ecological concept also known as EICA. Proposed by Blossey and Notzold (1995) this hypothesis proposes that because of release from enemy pressures, some invasive plants reallocate resources and rapidly evolve toward less defended but more vigorous types. |
| **Evolutionarily Stable Strategy (ESS) –** A strategy (often equated to a heritable or phenotypically plastic trait) or coexisting strategies (often equated to a polymorphic population or coexisting species) that when common in the population or community cannot be invaded by rare alternative strategies. |
| **Fecundity –** An ecological term that refers to the ability to reproduce. |
| **Genetic instability –** A term that refers to an increase in genomic alterations (e.g., mutation) in the majority of cells during division. |
| **Intravasation –** A term that refers to the invasion process of cancer cells entering the blood vessels or lymph vessels. |
| **Lag time –** A term used in both ecology and cancer studies to indicate the time that elapsed between initial establishment to proliferation. |
| **Metastasis –** A term that refers to the movement of a cancer lineage from a primary tumor to establish in another tissue. |
| **Metastatic potential –** A term that refers to the ability of cancer cells to leave the primary tumor and inhabit a distant organ. |
| **Oncogenesis –** A term that refers to the initial process of cancer initiation. |
| **Oncogenic cell –** A term that refers to a cell that expresses genes that potentially can cause cancer. |
| **Propagule pressure –** An ecological term that refers to the number of individuals released into a region. |

of "**metastatic potential**," but recent studies from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium have found that very early events in cancer are associated with predictable sets of so called "**drivers**" (Gerstung et al., 2020; but see concerns raised in Plutynski, 2021). Moreover, invasion in cancer studies depends strongly on context, including order of events and clonal composition (Birkbak and McGranahan, 2020). These characteristics contribute to disease state, metastatic potential, location of metastasis, and even response to therapy. The detailed studies of the behavior of a variety of human cancers promises to inform our understanding of genome level dynamics in the diversity of invasive species and provide insight for management.

Here, we aim to review the concepts related to the process of invasion and how they can be applied in parallel to the study of a broad variety of invasive taxa as well as a broad variety of metastatic (invasive) cancers. We then briefly review the applications of genomics technologies in these different fields, highlighting similarities, and differences. We emphasize that many findings in cancer research have not yet been replicated or uncovered in invasive species due to various limitations. We also emphasize the opportunities and need for research into questions that have not been answered in either invasive species or cancer. In order to identify these questions, we explore parallels in the recent summaries of 11 (Bock et al., 2015) and 14 (van Kleunen et al., 2018) open questions in the ecology and evolution of invasive species and 84 outstanding questions in cancer research (Dujon et al., 2021; see **Table 2** for summary).

## THE CONCEPT OF INVASION IN BIOLOGY

Definitions for biological invasions vary with the diverse aims of ecological studies (van Kleunen et al., 2015, 2018), but similar language and concepts have also been applied to cancer (Amend et al., 2016; de Groot et al., 2017; Ibrahim-Hashim et al., 2017; Pienta et al., 2020a; Dujon et al., 2021). The idea that cancer progression is an eco-evolutionary process has been discussed for over 50 years (Cairns, 1975; Nowell, 1976). de Groot et al. (2017) recently suggested that "studying cancer as an invasive species provides insight into the necessary phenotypic characteristics of the metastatic 'seed' and how those traits are selected for." They further describe similarities in migration to a "distant secondary habitat" through the use of "established **dispersal corridors**." In the case of cancers, these are blood vessels, lymphatics, and nerves (de Groot et al., 2017). Many ecological studies argue that the invasion process depends on the status of communities which may not be at an **Evolutionarily Stable Strategy (ESS)** for several reasons [e.g., empty niches, or anthropogenic induced changes; (McGill and Brown, 2007; Maron and Marler, 2008; Thuiller et al., 2010; Pintor et al., 2011)], which also applies in cancer (de Groot et al., 2017). Other studies have focused on the mechanisms that allow for individual species to be invasive (Richardson and Pyšek, 2006). This approach could be compared to the study of different successful cancer metastases (e.g., Turajlic et al., 2018), which has even been referred to as a speciation event (Capp and Thomas, 2020; Pienta et al., 2020a; Dujon et al., 2021). Several authors in ecology have also emphasized that the process depends on **propagule pressure** and time since introduction (Simberloff,

**TABLE 2 |** Similarities in approaches to study invasions in cancer and ecology [based on selected questions identified in Bock et al. (2015), van Kleunen et al. (2018), and Dujon et al. (2021)].

| Topic | Spread of cancer | Spread of invasive species |
|---|---|---|
| **(A) Questions about invasion that are not related to genomics** | | |
| Initiation | Do some organs develop more tumors? (seed and soil hypothesis) (Dujon #9) | Are some ecosystems more prone to invasion?* |
| | How does the risk of cancer initiation change with time? (Dujon #10) | How does risk of invasion of an ecosystem change with time?* |
| Environment | How does the tumor microenvironment drive tumor progression? What are the minimum resources necessary for the survival of cancer cells? Can targeting resources offer therapeutic opportunities? (Dujon #10, 24–26, 29) | How does habitat suitability benefit invaders? What mechanisms allow invasive plants to benefit from resource pulses? (#4 van Kleunen) What are the minimum resources necessary for habitat suitability? Can management of resources offer opportunities to control invasion? |
| | How does aging alter the tissue microenvironment thereby selecting for oncogenic cells? (Dujon #26) | How does disturbance change habitat suitability thereby promote invasion? Which alien species benefit from disturbance, and why? (#3 van Kleunen) |
| Enemies | What are the key dynamics in the interaction of cancer cells and the patient immune system? (Dujon #28) | Are invasive species less impacted by enemies? (enemy release hypothesis)* |
| Other questions | NA?* | What will be the future global distribution of alien plants? (#1 van Kleunen) What drives climatic niche shifts in the alien range? (#6 van Kleunen) How important are phylogenetic and functional diversity?(#8 van Kleunen) Do alien plants escape or recruit enemies at the range edges? (#9 van Kleunen) Do natives have novel weapons to resist alien invaders? (#10 van Kleunen) How important are mutualists compared with antagonists in driving invasions? (#11 van Kleunen) How frequent is rapid coevolution of aliens and natives? (#13 van Kleunen) |
| **(B) Questions that can be addressed with genomics** | | |
| Initiation | Are there differences in propagule pressure among cancers? Is it important?* | How important is propagule pressure? (#1 in Bock; #2 van Kleunen) |
| | What explains the existence and length of lag phases?* What molecular level processes differentiate benign versus malignant tumors? (Dujon #18) | What explains the existence and length of lag phases? (#7 in Bock; #5 van Kleunen) |
| | What is the cell of origin of a tumor? (Dujon #1) or cells of origin for metastases? | What is the source population of an invasive species?* |
| | Which subclones confer a fitness advantage? (Dujon #5) | Which genotypes are more fit?* |
| Phenotypic plasticity | What is the contribution of plasticity to cancer adaptations and how central is phenotypic plasticity in cancer and drug resistance during tumor progression and drug treatment? (Dujon #53) | Does phenotypic plasticity evolve in a predictable way? (Bock #8) Which strategies of adaptive plasticity are most frequent? (Bock #9) |
| | How does patient phenotypic plasticity (e.g., life-history trait adjustments and compensatory responses) affect evolution of cancer cells? (Dujon #14) | How does native species phenotypic plasticity (e.g., life-history trait adjustments and compensatory responses) affect evolution of invasive species?* |
| Heterogeneity | What is the role of tumor heterogeneity during metastasis (Dujon #21)? To what extent is tumor heterogeneity a cause or consequence of oncogenesis (Dujon #28)? What is the role of eco-evolutionary feedbacks between cancer cells and their tumor microenvironment? | What is the role of diversity of native species in dispersal/invasion? To which extent is genetic diversity cause/consequence of invasion? What traits or trait combinations, if any, best predict invasion success? (Bock #5; van Kleunen #7)? Are trait changes in introduced populations really adaptive? (#12 van Kleunen) What is the genetic basis of observed phenotypic evolution? (#14 van Kleunen) |
| Models | Can we build genetic models that forecast tumor evolution? (Dujon #60 and #61) | Can we build genetic models that predict invasiveness?* |
| Immune system | What is the role of the immune system in somatic evolutionary trajectories leading to cancer? (Dujon #39) | Do invasive plants grow faster and/or produce more seeds but become less well-defended against enemies (EICA Hypothesis).* |
| | How can we best harness a patient's immune system to tackle cancer evolution? (Dujon #40) | How can we choose the best biocontrol agents?* |
| Other questions | NA? | Why does hybridization sometimes result in increased colonization success and sometimes does not (Bock #2)? Whether the accumulation of deleterious mutations limits invasions and/or if compensatory mechanisms reduce the severity of expansion load (Bock #4)? Why do some invaders exhibit strong local adaptation and others do not (Bock #6)? Is the genetic architecture of invasiveness traits different from that of other traits that differentiate natural populations or species (Bock #10)? To what extent are genes "re-used" during the evolution of invaders (Bock #11)? |

**Not specifically listed in these references.*
*# = the question number as defined in the referenced publication.*

2009; Bock et al., 2015), which is similar to the concept of "billions of failures" in circulating cancer cells that lead to very few successful metastases (de Groot et al., 2017; Tissot et al., 2019). Likewise, many studies of invasive species report that improved **fecundity** could contribute to their rapid spread and population growth in the invaded range (Bock et al., 2015), which has obvious parallels in metastatic cancers (Lloyd et al., 2016; Vittecoq et al., 2018). In both ecology and cancer, the goal is to understand the causal processes involved in the transport, establishment, spread, and eventual adaptations of invasive species. However, many experimental studies of species invasions are limited in scope, because they provide only a single snapshot of native and invasive populations. When genomics approaches have been used, only a very small representation of genome level mechanisms and dynamics were assayed (Bock et al., 2015; Richards et al., 2017; Paun et al., 2019; Richards and Pigliucci, 2020; Mounger et al., 2021a).

Although limited in genomics prowess, ecological studies across a tremendous diversity of species have developed an array of theoretical frameworks to understand the process of invasion and examine fundamental questions in ecology and evolution (Gurevitch et al., 2011; Bock et al., 2015; van Kleunen et al., 2018). There is intense pressure to understand the process of biological invasions due to their ecological and economic effects (Simberloff et al., 2013; Bellard et al., 2016b; Lodge et al., 2016; Diagne et al., 2020). These effects arise from three ecological characteristics of a successful species invasion: rapid increase in population, local dominance or monoculture, and rapid range expansion (Gurevitch et al., 2011). Research has resulted in many proposed causal explanations of these invasion dynamics, including **propagule pressure** (Simberloff, 2009; Britton and Gozlan, 2013), **biotic resistance** (Levine et al., 2004; Nunez-Mir et al., 2017), and **evolution of increased competitive ability** (Blossey and Notzold, 1995; Rotter and Holeski, 2018). The variety of support (and lack thereof) for these explanations makes it clear that no single factor can explain all biological invasions or contribute to all of them (Catford et al., 2009; Gurevitch et al., 2011; van Kleunen et al., 2018). However, efforts to synthesize these explanations have resulted in valuable conceptual frameworks that relate ecological and evolutionary processes to the steps and barriers of the invasion process.

These conceptual frameworks describe species invasion as a process with several steps, or filters, through which non-native species introduced to a new range must pass before exhibiting the characteristics of invasiveness (Lodge, 1993; Rejmánek, 2000; Richardson et al., 2000; Blackburn et al., 2011; van Kleunen et al., 2018). A species must be (1) **transported** and (2) **introduced** to the novel range via natural or human-assisted dispersal. Then, the species needs to (3) survive and become **established** in the novel range. Finally, an invasive species is (4) able to reproduce and **spread** (**Figure 1**; Theoharides and Dukes, 2007; Blackburn et al., 2011; Lloyd et al., 2017; van Kleunen et al., 2018).

The metastatic cascade follows closely the same steps of how invasive species disperse, colonize, and spread with the exception that the first step of "transport" is typically broken down into two parts (green boxes in **Figure 1**; Chen and Pienta, 2011; Lloyd et al., 2016). Metastasis includes: (1) **intravasation** (invasion into the bloodstream or lymphatics system), (2) **circulation** and evasion of the immune system, (3) **extravasation** (exiting the bloodstream), (4) **establishment and angiogenesis**, and (5) **spread** (accessing and establishing vasculature; Paterlini-Brechot and Benali, 2007; Gatenby et al., 2009; Hapach et al., 2019). Intravasation involves cancer cells from an established tumor entering or falling into the bloodstream. Such cells are not a random subset of the tumor's cancer cells, and location within the tumor will likely matter (Lloyd et al., 2016, 2017; Ibrahim-Hashim et al., 2017). Those at the tumor's edge will have access to larger, normal blood vessels while those in the interior will experience the disorganized and poorly perfused vasculature induced by angiogenesis. The cancer cell's characteristics will also matter. For instance, the epithelial to mesenchymal transition in cancer cells generates a more motile phenotype capable of squeezing between cells including those forming the walls of blood vessels (Barriere et al., 2015). So, while intravasation involves accidental dispersers (Joosse et al., 2015), the cancer cells that enter the bloodstream as **circulating tumor cells (CTCs)** are a weighted average of cancer cell types within the tumor.

Ecological studies offer additional conceptual frameworks that could be explored more thoroughly with genomic approaches in both invasive species and cancers (see **Table 2B**). Forecasts of invasion risk have been made based on the relationship between phylogenetic distance between the invaders and members of the invaded community (van Kleunen et al., 2015, 2018). A related concept is that species may be "pre-adapted" if the recipient environment is a close match to the native environment and the breadth of the native range may be a good predictor of this possibility (Bossdorf et al., 2005; Bock et al., 2015). These predictions based on phylogenetic distance among species can be argued to support multiple outcomes leading to "Darwin's naturalization conundrum" (Diez et al., 2008; Thuiller et al., 2010). For example, "Darwin's naturalization hypothesis," argues that invaders that are phylogenetically unrelated to local communities should be more successful because they can exploit unfilled ecological niches in native communities (Rejmánek, 1996; Thuiller et al., 2010). On the other hand, closely related species might share similar pre-adaptations to local environmental conditions or have similar biotic or abiotic requirements (Thuiller et al., 2010). Regardless, phylogeny does not always reflect trait differences or niche differentiation, and often the importance of these different components of invasion potential are unknown (Thuiller et al., 2010; van Kleunen et al., 2015, 2018). Recent application of genomic techniques to trace the "life history" of cancers have found parallels in a diversity of cancer types (Nik-Zainal et al., 2012; Turajlic et al., 2018; Gerstung et al., 2020), but this type of evolutionary ecology framework has not yet been fully explored in the study of cancers.

Many studies in both ecology and cancer have demonstrated that in addition to the importance of pre- or post-invasion sources of genetic differences, phenotypic plasticity can be important. A rich literature in evolutionary ecology has explored several different scenarios for how invasive species may benefit from phenotypic plasticity, some of which were outlined by Richards et al. (2006). Plasticity may allow for invasives to be a (1) "Jack-of-all trades" with positive fitness across many habitat

types; (2) "Master-of-some" with highly positive fitness in the most favorable habitats and conditions, or (3) "Jack-and-Master" that combines both attributes. However, increased plasticity does not always translate into positive fitness outcomes (Levis and Pfennig, 2016; Matesanz et al., 2017; He et al., 2021). In fact, several meta-analyses (Van Buskirk and Steiner, 2009; Davidson et al., 2011; Palacio-López and Gianoli, 2011; Arnold et al., 2019) have not supported the hypothesis that plasticity contributes to the success of invasive species. In either case, the mechanisms underlying this plasticity will be mediated by genetic and non-genetic molecular level processes (Richards et al., 2010; Nicotra et al., 2010; Herman and Sultan, 2011, 2016; Banta and Richards, 2018). While the fine molecular details of plastic responses have not yet been assessed in any species (Richards et al., 2017; Bock et al., 2018; Laitinen and Nikoloski, 2019; Richards and Pigliucci, 2020; Sommer, 2020; Mounger et al., 2021a), the potential to do so is currently greatest in cancers.

Another important concept that has been explored in evolutionary ecology is the role of propagule pressure, or the rate of arrival of non-native individuals. Propagule pressure can be important not only to the initial step of introduction, but it can influence survival by overwhelming stochastic processes, and increasing survival, reproduction, and competitive dominance. High propagule pressure can enhance genetic diversity permitting the invasive species to persist and thrive in a new environment (Simberloff, 2009; Rius et al., 2015b). Invasive species often exhibit a lag time, where extended periods of time pass between initial establishment and later development of invasive characteristics (Simberloff, 2009; Aikio et al., 2010; Bock et al., 2015; van Kleunen et al., 2018). In ecology, the length of this lag time remains unpredictable and the mechanisms at work remain unknown, but rapid evolution has occurred on the scale of <50 years, and evidence shows that local adaptation occurs in invasive species (Bock et al., 2015). Similarly, disseminated cancer cells (DTC) that arrive at and survive in a novel organ may exhibit long lag periods before expanding from micrometastases into clinically detectable metastatic tumors (Birkbak and McGranahan, 2020).

Recent evidence from the Pan Cancer Atlas has traced the life-history of thousands of cancers and found the lag time from so called "driver events" to detection can be on the order of years to decades (Nik-Zainal et al., 2012; Alexandrov et al., 2013b; Gerstung et al., 2020). Extensive research has detailed several components of the metastatic process. The quantity of CTCs with potential to seed new tumors depends on survival while passing through the bloodstream (**Box 1: Figure A**). This involves tolerating potentially destructive shearing forces from moving swiftly through vasculature, as well as avoiding detection and mortality from the immune system in the blood (Lloyd et al., 2016). Additional characteristics such as immune evasion and the ability to be compressed may enhance the chances of a cancer cell surviving in the blood. Authors have suggested that several cancer cells traveling together as a raft (Aceto et al., 2014), or unusually large cancer cells such as those in a polyaneuploid state (polyaneuploid cancer cells, PACCs) may be able to circulate longer and more successfully (Pienta et al., 2021). However, regardless of characteristics, the half-life of a CTC remains unknown, yet is vitally important. Blood circulates throughout the human body on average every 45 seconds, and the number of times a CTC can circulate influences their number as well as their likelihood of entering other tissues. CTCs can reach 1–10 per ml of blood (Yu et al., 2011; Alix-Panabières and Pantel, 2021), and the number of CTCs in the blood correlates with progression free survival (5 or more CTCs per 7.5 ml of blood represents a poor prognosis for breast or prostate cancer patients; Rack et al., 2014; Pantel and Alix-Panabières, 2019).

In addition to these characteristics that might contribute to the success of CTCs, only a small fraction of CTCs are able to exit a blood vessel and enter a novel tissue or novel location in the same tissue type. Actin dynamics within a cancer cell can permit pseudopodia facilitating motility and the ability to move through cell junctions (diapedesis) (Castro-Giner and Aceto, 2020). The establishment of a successful metastasis from one or several of such DTCs represents yet another hurdle. Several characteristics of the DTC may favor success albeit it is still a very small probability. Actin mechanics can be critical for degrading and constructing extra-cellular matrices (Kumar and Weaver, 2009). The establishing DTC must overcome additional threats from what can be a tissue specific immune response. To avoid failure, the DTC or emerging micrometastasis must overcome Allee effects (Johnson et al., 2019), and access and establish vasculature (angiogenesis) (Amend et al., 2016; Lloyd et al., 2016, 2017).

Despite these hurdles that are difficult to trace, the metastatic cascade is a simpler and perhaps more accessible microcosm for the evolutionary ecology of invasive species. The patient is the entirety of the system, i.e., the entire planet for the cancer (Pienta et al., 2020a). All the living cells within the patient, including the cancer cells and normal cells are relatively similar in size. The pathway to invasion in cancer is straightforward, occurring through the blood or lymphatics. The cancer cell will face roughly the same supply of resources, types of resources, and threats from the immune system. However, the different organs and tissues of the body differ structurally, functionally, and metabolically in ways that influence their susceptibility to metastases (Schild et al., 2018). Like in invasion ecology, the heritable characteristics of the metastasizing cancer cells matter (de Groot et al., 2017; Pienta et al., 2020a). They can carry adaptations for dispersal and pre-adaptations for surviving the rigors of dispersal and avoiding the immune system (Hanahan and Weinberg, 2011; de Groot et al., 2017). Similarly, ecological species invasions can be facilitated by dispersal adaptations such as burrs on seeds that stick to the fur of animals (or artificial surfaces) or dispersal events prompted by over-crowding. Also, there is often an association between the habitat characteristics of the donor and recipient locations (van Kleunen et al., 2018). This aligns with Paget's (1889) seed-and-soil hypothesis for the relationship between the donor tumor's tissue type and the recipient tissue where metastases are likely to occur. Some authors have even suggested that exosomes released by cancer cells in the primary tumor "prepare the soil" for the metastatic "seeds" (Rodrigues et al., 2019). A reasonable comparison

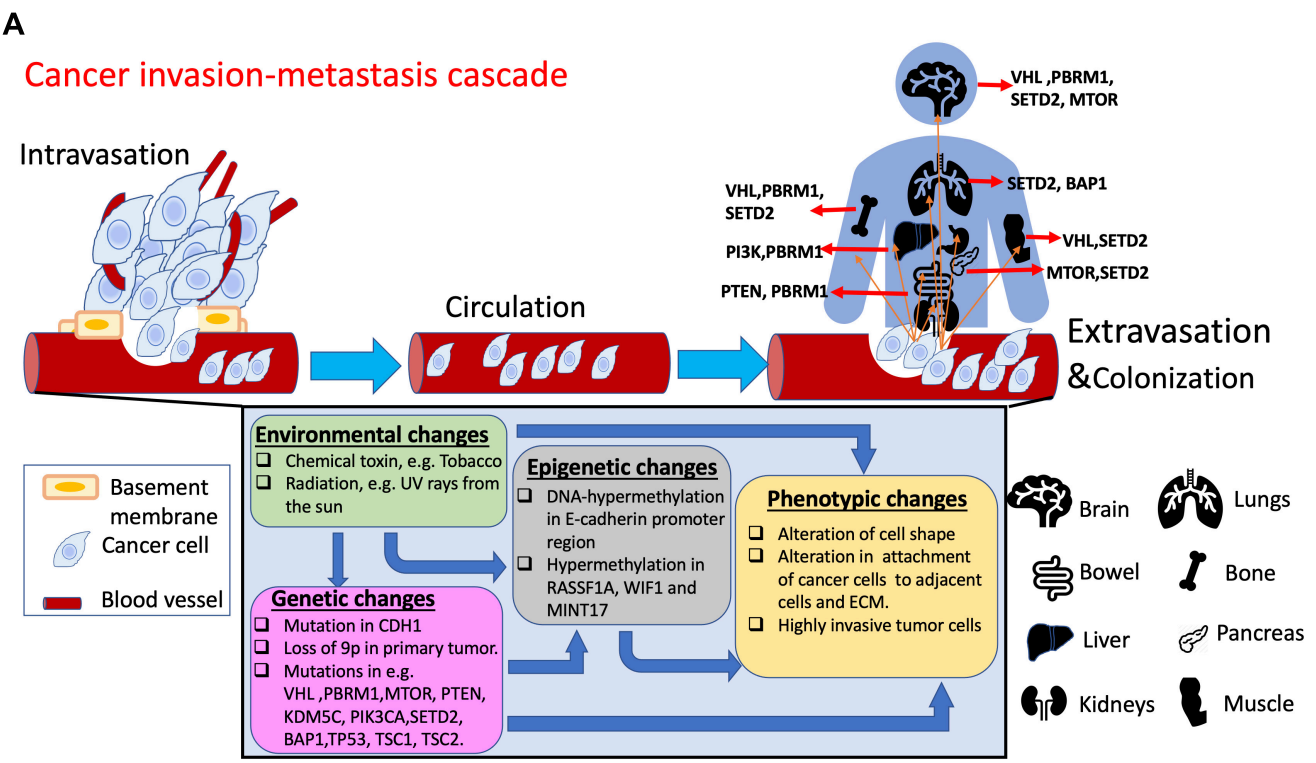
in ecological invasions could be human habitat disturbances and constructs that favor the invasion of numerous species (van Kleunen et al., 2018).

Upon successful establishment, the cancer cells can evolve further adaptations to successfully exploit their novel environment. From the perspective of the emerging field of invasion genomics, principles from invasion ecology in natural systems offer a conceptual framework for metastases. In return, the extensive, replicated opportunities for measuring DNA sequence, epigenetic markers, gene expression, and heritable characteristics of cancer cells means that oncology offers unique opportunities for evaluating and testing the genomics of species invasions (e.g., **Box 1: Figure A**; Turajlic et al., 2018).

## GENOMICS OF INVASION

In both species level ecology and cancer, one major goal is to identify how genomic level processes translate into the ability of the organism to respond to complex environmental conditions. The genomic mechanisms that underlie invasions are particularly intriguing because of classic evolutionary theory that predicts how the ability to respond to environmental challenges rests on heritable phenotypic variation which is presumably genetically based. The fact that invasions by definition have been assumed to result from just a few individuals creates a so-called "genetic paradox" for understanding the success of invaders and their adaptations to new habitats (Allendorf and Lundquist, 2003;

**BOX 1 |** The invasion processes in cancer and ecology share commonalities but there are also important differences. The cancer metastasis and successful invasion process consists of a sequence of steps that are like the steps of ecological invasion (see **Figure 1**). Here, we highlight examples of genetic and epigenetic mechanisms involved in invasion in cancer and two different ecological systems. **(A)** Cancer invasion. Cancer cells invade the basement membrane and enter the blood vessels (intravasation), circulate in the blood and reach a distal organ (e.g., the liver). Genetic as well as epigenetic alterations govern cancer metastasis. One of the most studied alterations is the reduction of E-cadherin protein expression, which is responsible for the adhesions between cells. Mutations have been found in the CDH1 gene that codes for E-cadherin, as well as DNA hypermethylation. Specific site of metastasis has been associated with genomic driver mutations that occurred in the primary tumor: examples from Turajlic et al. (2018) are depicted where metastases from primary kidney tumors metastasized to lung, bone, liver, brain, pancreas, and muscle (adrenal, parotid, thyroid glands, skin, and soft tissue not shown). **(B)** Cane toad invasion. A total of 101 cane toads were introduced to Australia in 1935 from Central and North America. The invasion traveled from the northeast to southern and northwestern regions of Australia resulting in heritable differences in physiological, morphological, and behavioral traits. After already surviving a bottleneck from the initial invasion, genetic diversity declined between the initial site of establishment and the leading edge of the invasion (Selechnik et al., 2019). One potentially important alteration is that genes involved in metabolism and immune function were upregulated (Rollins et al., 2015). Experimental manipulations also support heritable epigenetic changes at the SCNN1G gene could be involved in the response to predators (Sarma et al., 2020, 2021). **(C)** Japanese knotweed invasion. This plant was first introduced from Japan to Europe in the 1840s and then to North America sometime before 1873 (Del Tredici, 2017). The invasive knotweed *Reynoutria japonica* has been reported to be a single widespread genotype, however, the molecular characterization of this species has been limited and cannot exclude potentially important single nucleotide polymorphisms (Mounger et al., 2021a). On the other hand, epigenetic markers have been associated with differences in habitat and climate (Richards et al., 2012; Zhang et al., 2016).

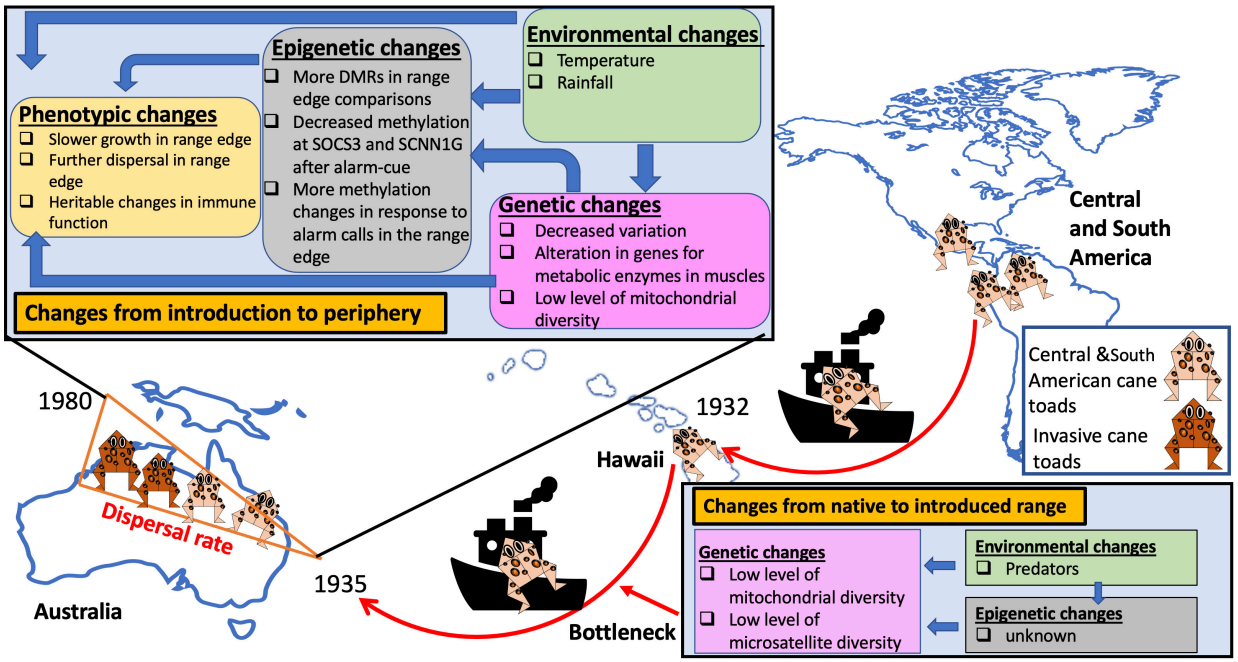



*(Continued)*

**BOX 1 |** (Continued)

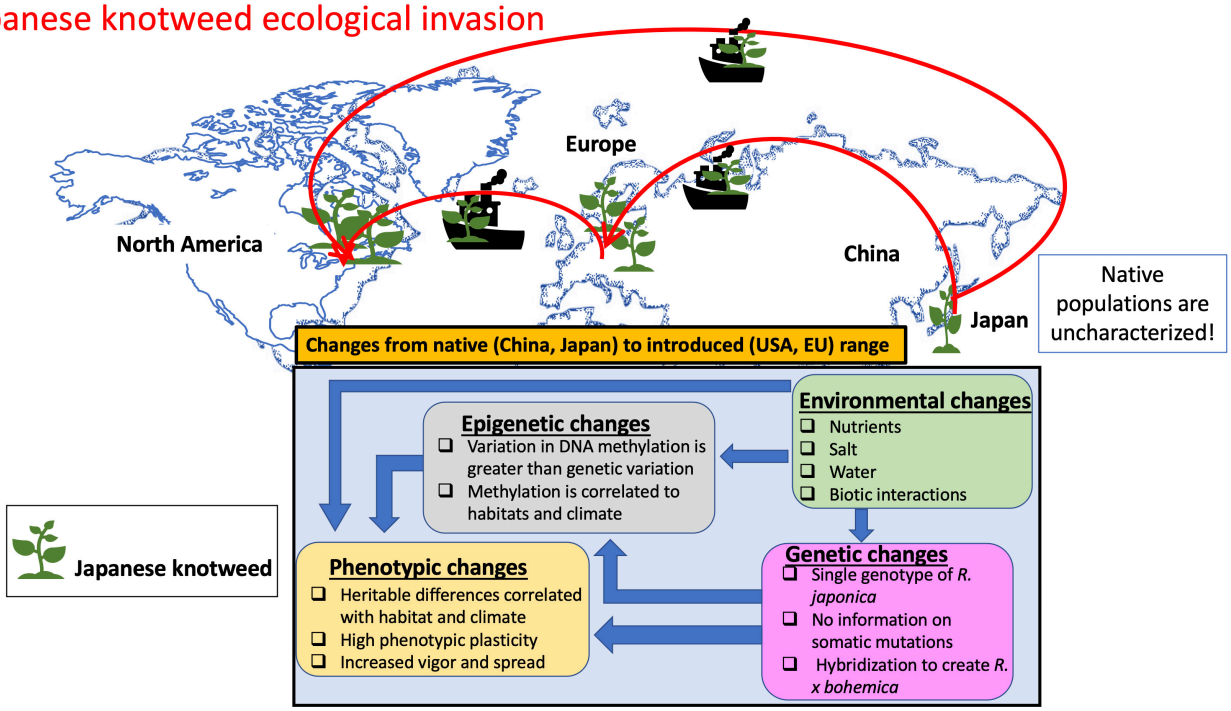
**B**

## Cane toad ecological invasion




**C**

## Japanese knotweed ecological invasion



Estoup et al., 2016). This has been a mystery in both ecology and cancer research with several hypotheses about how the invaders overcome the negative consequences of limited genetic variation (Baker and Stebbins, 1965; Nowell, 1976; Vogelstein et al., 2013; Estoup et al., 2016).

In this context, both invasive species studies and cancer studies have largely focused on the importance of sequence based differences or mutations (Alexandrov et al., 2013b, 2020; Vogelstein et al., 2013; Bock et al., 2015). However, evolutionary responses to challenging environmental conditions rely on heritable phenotypes, regardless of the underlying mechanisms of inheritance (Jablonka and Lamb, 1998, 1999; West-Eberhard, 2005; Banta and Richards, 2018: Bonduriansky and Day, 2018). We now have evidence that the structural and functional dynamics of genomes along with a variety of epigenetic and other non-genetic effects can contribute to heritable variation and thus to adaptation and cancer metastasis (Feinberg et al., 2006; Jablonka and Raz, 2009; Johannes et al., 2009; Feinberg and Irizarry, 2010; Timp and Feinberg, 2013; Richards et al., 2017; Bonduriansky and Day, 2018; Kooke et al., 2019; Richards and Pigliucci, 2020).

## Discoveries and Limitations of Genomic Studies of Diverse Invasive Species

Genetic markers have been used to identify the genetic make-up of invasive populations, and understand how genome level processes contribute to invasion of diverse natural ecosystems (Bock et al., 2015; Colautti and Lau, 2015; Dlugosch et al., 2015; Rius et al., 2015b; Mounger et al., 2021a). Studies in the native and introduced ranges have reported that many invasive populations undergo only modest reductions in genetic variation due to multiple introductions (Stepien et al., 2005; Dlugosch and Parker, 2008a; Snyder and Stepien, 2017; Flucher et al., 2021), hybridization (Fitzpatrick et al., 2009; Scascitelli et al., 2010; van Riemsdijk et al., 2018, 2020; Quilodrán et al., 2020), or Allee effects that result from reverse density dependence or cooperation (Kramer and Sarnelle, 2008; Aikio et al., 2010; Rius et al., 2015b). For example, sequences of mitochondrial DNA revealed multiple invasion sources for both dreissenid mussels and goby species of fish, and that this diversity was correlated to rapid spread and colonization success in a variety of habitats (Stepien et al., 2005). Further, invasive populations of zebra mussels, quagga mussels, round gobies, tubenose gobies, and Eurasian ruffe (another fish species) that have established in the Great Lakes had as much or greater genetic diversity as native populations (Stepien et al., 2005; Snyder and Stepien, 2017). Recently, reduced representation sequencing (Narum et al., 2013) has provided much more power to inform studies of invasion by demonstrating, for instance, the absence of strong population structure which could indicate repeated human-assisted dispersal across the invaded range such as in the pavement ant (*Tetramorium immigrans*) (Zhang et al., 2019).

On the other hand, lower diversity in invasive populations may reflect that there was a higher diversity of founding genotypes initially, and selection in the novel habitat filtered out unfit individuals (Dlugosch and Parker, 2008b; Vandepitte et al., 2014). Other studies argue that the process of genetic bottlenecks can purge deleterious alleles, reveal beneficial cryptic variation or create new beneficial interactions among genomic elements (Colautti and Lau, 2015; Dlugosch et al., 2015; Stapley et al., 2015; Estoup et al., 2016; van Kleunen et al., 2018). The invasive brown rat (*Rattus norvegicus*) is a globally successful invader, but recent studies in China discovered that local populations had experienced severe bottlenecks and then rapidly differentiated since establishment in the 1970s, including new alleles associated with lipid metabolism and immunity genes (Chen et al., 2021).

Despite such bottlenecks, loss of diversity measured by molecular markers does not necessarily reflect loss of quantitative trait variation (Dlugosch and Parker, 2008b; Estoup et al., 2016). A series of studies revealed that rapid phenotypic evolution facilitated the invasion of the widespread red macroalga *Agarophyton vermiculophyllum* despite the fact that the species experienced a severe genetic bottleneck and increased through clonal spreading (Krueger-Hadfield et al., 2016; Sotka et al., 2018; Flanagan et al., 2021). The European starling (*Sturnus vulgaris*) in North America was founded by only ∼180 individuals, but local populations seem to have evolved rapidly and now show the signature of only a moderate population bottleneck (Hofmeister et al., 2021). European starlings in North America show higher levels of genetic diversity than invasive populations in Australia or South Africa (Bodt et al., 2020). This is somewhat surprising since the invasion in Australia occurred across multiple introduction sites and the pattern of rapid differentiation appears to be explained by distance instead of response to environment (Rollins et al., 2016; Stuart et al., 2020).

Most genomics studies of invasive species lack the resources required for understanding the single base pair resolution of how specific genome level differences might translate into function and the species' success. However, transcriptomic studies can now be conducted in almost any system, providing a measure of variation in gene expression at the level of mRNA, which contributes to the formation of proteins, cellular phenotypes and ultimately the organism's phenotype (Alvarez et al., 2015). A few studies have identified candidate genes that were differentially expressed in invasive populations (Hodgins et al., 2013; Bock et al., 2015). Studies of populations of the Argentine ant (*Linepithema humile*) discovered consistent differences among invaded compared to native populations in expression of genes related to biogenic amines (which modulate behavioral traits like foraging and aggression) and immune function. Unfortunately, they could not associate these expression differences with behavioral differences in their study. Furthermore, interpretation of the functional relevance of some of the differentially expressed genes was limited by the need for better annotation (Felden et al., 2019).

Hodgins et al. (2015) compared transcriptomes across 35 species of plants in the Asteraceae, including six major invasive species. They found no support for the idea that there was consistent selection on genes that contributed to invasiveness [but see opposite results with a similar approach in the invasive green crab, *Carcinus maenas* (Tepolt and Palumbi, 2020)]. In a rare comparison of sequence variation and expression variation, a study of two independent invasions of the Pacific Oyster (*Crassostrea gigas*) into the North Sea found little overlap between

differentially expressed genes and outlier loci. This suggested that differential gene expression did not necessarily correlate with changes in allele frequencies (Wegner et al., 2020). However, as is common in ecological genomics, this study suffered from limited annotation of the transcriptome and limited coverage of the genome (using reduced representation RADseq). In general, identifying underlying molecular level "drivers" is so far limited to very few studies across widely diverse taxa (Bock et al., 2015).

In addition to using transcriptomics approaches, several authors have argued that epigenetic mechanisms could be particularly important for invasive species (Ardura et al., 2017; Hawes et al., 2018; Marin et al., 2020; Mounger et al., 2021a; but see Eckert et al., 2021). The case has been made particularly for those invasive species that are clonal or have low genetic diversity. Epigenetic mechanisms could provide a non-genetic source of heritable variation (Verhoeven and Preite, 2014; Douhovnikoff and Dodd, 2015; Liebl et al., 2015; Rollins et al., 2015; Richards et al., 2017; Sarma et al., 2020, 2021; Mounger et al., 2021b). In the last 10 years, there has been an explosion of studies of natural populations and ecological experiments that provide some level of information about how epigenetic mechanisms (mainly DNA methylation) may contribute to organismal responses to environmental challenges (Jablonka, 2017; Richards et al., 2017; Richards and Pigliucci, 2020; Stajic and Jansen, 2021). But so far these ideas and approaches have rarely been applied to the understanding of invasive species (Hawes et al., 2018; Marin et al., 2020) and they are almost universally limited in scope (Paun et al., 2019; Richards and Pigliucci, 2020; Mounger et al., 2021a).

The recent work in cane toads (*Rhinella marina*) provides one excellent example of integrating genomic, transcriptomic, and epigenomic approaches to understand the process of invasion (Rollins et al., 2015; Selechnik et al., 2019; Sarma et al., 2020, 2021). A substantial bottleneck occurred during the introduction of only 101 cane toads into Queensland, Australia in the 1930s (Shine, 2018; **Box 1: Figure B**). At present, the toads at the edge of their range are less genetically diverse than those at the initial site of introduction, but show a wide variety of heritable differences in physiological, morphological, and behavioral traits (Phillips et al., 2008; Rollins et al., 2015; Selechnik et al., 2019). In addition, this team of researchers suspected that toads at the invasion front encounter more abundant predators than at the original site of introduction, which may contribute to higher mortality rates and select for larger toxin glands. By radio-tracking toads, researchers were able to see that some toads moved long distances almost every night in the first 2 years, and inheritance of rapid dispersal became spatially sorted (Shine et al., 2011). Rollins et al. (2015) argued that this resulted in assortative mating among individuals at the front of the invasion between fast-moving individuals which reinforced the evolution of this trait (Shine et al., 2011). By measuring genome wide levels of gene expression with RNAseq, they also showed that metabolic enzymes were upregulated at the invasion front and that many of the most highly differentially expressed genes involved energy production, immune function, and parasite resistance (e.g., PSME4 and RASGEF1B) (Rollins et al., 2015).

Remarkably, these researchers took advantage of this invasion gradient to experimentally examine the potential role of epigenetic mechanisms as well (Sarma et al., 2020, 2021). They exposed tadpoles to alarm cues and found elevated cortisone levels as well as decreased methylation at the suppressor of cytokine signaling 3 (SOC3) and the Sodium Channel Epithelial 1 Subunit Gamma gene (SCNN1G) genes. They further tested the idea that DNA methylation drives this pattern by manipulating methylation levels with the drug zebularine but could not associate changes in DNA methylation to the promoter region of the glucocorticoid (GC) receptor gene (NR3C1). However, they did find differences in single cytosine methylation in the promoter region of SOCS3, which may be involved in predator avoidance behavior (Sarma et al., 2020). This team then examined the inheritance of changes in methylation by running a breeding experiment. They showed that some shifts in DNA methylation in response to alarm cues were inherited by the next generation. In particular, they showed demethylation within SCNN1G, which regulates sodium in epithelial cells and may help to maintain the epidermis (Sarma et al., 2021). While this series of studies has not dissected every molecular detail of the invasion response, it is unparalleled in their exploration of various levels of response. This research team demonstrated the wide variety of questions that can be addressed with genomics approaches.

In the last few decades, we have gained increased data about the genetic structure of a variety of invasive species and how genetic variation is distributed on the landscape (Bock et al., 2015), but most of our information is limited to markers distributed across the genome and very few studies can evaluate the whole genome. Many studies have only described patterns of diversity and are limited in their ability to address underlying adaptive processes (Rius et al., 2015a,b). Rius et al. (2015a) report that the application of next generation sequencing (NGS) to invasive species started in 2008 and by 2015 resources had been developed for many species. NGS had even identified candidate genes like the detoxification gene cytochrome P450 and other stress related genes. However, how the translation of DNA sequence to phenotypes unfolds through the invasion process requires much more fine scale dissection of the entire genome and other molecular level processes over time (see e.g., Bock et al., 2018; reviewed in Pigliucci, 2010; Keller, 2014; Müller, 2017; Bonduriansky and Day, 2018; Richards and Pigliucci, 2020). The increasing application of sequencing and other "omics" technologies within appropriately designed experiments promises to provide more powerful insight into the molecular mechanisms underlying responses to selection and adaptation (Alvarez et al., 2015; Rius et al., 2015a,b; Mounger et al., 2021a), but it is yet unclear how far we can go with this approach and in how many unique species. In this context, cancer genomics studies provide some important insights.

On the other hand, the diversity represented among invasive species provides information about the potential for novel function, particularly in organisms with extreme phenotypes (Castoe et al., 2013; Bock et al., 2018). A study of invasive Burmese pythons before and after a major freeze event in Florida in 2010 found evidence for directional selection in genomic regions enriched for genes associated with thermosensation, behavior, and physiology. Several of these genes were linked to regenerative organ growth, which modulates feeding and fasting responses in pythons (Card et al., 2018). In addition, ecological

experimental approaches can be quite creative in testing for the importance of some of these processes by combining genomics approaches with, for example, the creation of synthetic hybrids (Rosenthal et al., 2002; Rieseberg et al., 2003; Lai et al., 2006; Whitney et al., 2015; Nieto Feliner et al., 2020; Irimia et al., 2021), and synthetic or recent polyploids (Yoo et al., 2014; Nieto Feliner et al., 2020; Paape et al., 2020; Shan et al., 2020).

## Are Clonal Plant Species a Particularly Useful Comparison to Cancer?

Some of the world's most successful invasive plants are thought to spread by clonal reproduction (e.g., Japanese knotweed **Box 1: Figure C**), which at first brush might seem like a good analogy for invasion in cancer considering that cancers arise within a host who has the same genotype. Mounger et al. (2021a) recently reported that clonal plants are potentially over-represented among invasive plant species. This is surprising because asexual reproduction is predicted to result in slower rates of evolution. But clonality may also be adaptive under the circumstances faced by invasive species and serve as useful subjects to investigate how a single genome can respond to a myriad of environments (e.g., Geng et al., 2007; Verhoeven et al., 2010; Gao et al., 2010; Richards et al., 2012; Shi et al., 2018; Chen et al., 2020). A recent study in the perennial sunflower, *Helianthus tuberosus*, provided a powerful combination of approaches to demonstrate that there had been selection for the ability to increase clonality (Bock et al., 2018). The authors compared populations of ancestral lineages with invasive lineages and found support for increased ability to respond to well watered conditions by producing more clonal propagules. As such, this is one of the first studies to demonstrate the process of genetic accommodation during invasion (sensu West-Eberhard, 2005; see also Sultan, 2015; Levis and Pfennig, 2016). In this study, the researchers were able to link the genomic mechanisms of hybrid vigor and two specific QTL to the increased ability to respond to water content in the invasive habitat. We know of no study where the genomic level processes that underlie the success of entirely clonal lineages have been fully explored. Genetic variation that arises from somatic mutations in natural clonal lineages, albeit low, cannot be excluded since several studies have reported that high rates of somatic mutation may allow asexual species to maintain abundant genetic variation and adapt to changing environmental conditions (reviewed in Schoen and Schultz, 2019; see also discussions in Chen et al., 2020; Robertson et al., 2020).

Japanese knotweed (*Reynoutria japonica* aka *Fallopia japonica*) is one of the most well−known cases of an invasive clonal plant. A single octoploid clone of *R. japonica* has spread aggressively through a broad range of habitats in temperate Europe and North America (**Box 1: Figure C**; Beerling et al., 1994; Bailey and Conolly, 2000; Grimsby et al., 2007; Gerber et al., 2008; Bailey et al., 2009; Richards et al., 2012; Zhang et al., 2016). Unfortunately, not much is known about the levels of diversity in the native populations of China and Japan. In the United States, replicates of the same clone of *R. japonica* collected from different habitats had different DNA methylation patterns even after they were grown in a common garden in New York (Richards et al., 2012). Another study across central Europe showed that individuals from different populations of this same *R. japonica* clone harbored significant epigenetic and phenotypic variation which was associated with climate (Zhang et al., 2016). However, both studies were based on anonymous molecular markers (AFLP and methylation sensitive AFLP) which only survey a small portion of the genome. They cannot detect single DNA base changes even in the surveyed fragments (Schrey et al., 2013; Paun et al., 2019). A recent survey of the same samples in the United States populations suggested that within this *R. japonica* clone there were most likely some single nucleotide polymorphisms (Robertson et al., 2020; see also VanWallendael et al., 2020), but whether any of these polymorphisms are functional remains to be evaluated.

As in the knotweed studies, the low genomic resolution of studies of most organisms precludes pinpointing the actual accrual of sequence and methylation polymorphisms and therefore isolating the importance of genetic and epigenetic variation (Richards et al., 2017; Paun et al., 2019; Naciri and Linder, 2020). The whole genome sequencing studies that have been done (almost entirely in model species) reveal the importance of genomic redundancy, largely resulting from multiple episodes of whole genome duplication (polyploidy) followed by reduction processes (Doyle et al., 2008; Wendel et al., 2016), which play a major role in diversification and adaptation in plants and some animals (Van de Peer et al., 2017). Whole-genome studies in the model plant *Arabidopsis thaliana* (as in cancers) show that novel epigenetic variation can be dramatically shaped by *de novo* sequence mutation. For example, studies have found that single nucleotide polymorphisms can change the methylome by modifying a methyl transferase or a nucleotide context where methyltransferases act (Becker et al., 2011; Timp and Feinberg, 2013; Dubin et al., 2015; Feinberg et al., 2016; Sasaki et al., 2019).

The relevance of somatic mutations has been clearly documented in cancers (Nik-Zainal et al., 2012; Alexandrov et al., 2013b, 2020; Gerstung et al., 2020), and in cancer metastases (e.g., Turajlic et al., 2018). Mutation could contribute to the rapid generation of genetic or epigenetic variation in natural clonal lineages of plants, and in organisms more generally (Vonholdt et al., 2010; Exposito-Alonso et al., 2018; Hawkins et al., 2019; Schoen and Schultz, 2019; Yoder and Tiley, 2021). But it may be unclear how comparable mutation in cancers is to natural populations of plants and animals. Studies across plant species have reported a range of mutation rates from e.g., $7 \times 10^{-9}$ per base per haploid genome per generation in *Arabidopsis thaliana* lines (Ossowski et al., 2010; Exposito-Alonso et al., 2018) and peach (Xie et al., 2016) to $4 \times 10^{-8}$ in long lived poplar and oak species (Schmid-Siegert et al., 2017; Hofmeister et al., 2020). Mutation rates across a diversity of animal species ranged from $3.6 \times 10^{-9}$ in bumblebee to $1.5 \times 10^{-8}$ in chimpanzee (Yoder and Tiley, 2021). This is roughly comparable to the average generational mutation rate for single-base substitutions in humans $1–1.5 \times 10^{-8}$ (Rahbari et al., 2016). Therefore, studies in human cancer could provide insight into the mechanisms that underlie rapid organismal response more generally.

Unlike in most species, cancer studies have unsurpassed power to document how mutation rate depends on location and nucleotide context in the genome as well as tissue types (Alexandrov et al., 2013a, 2020; Rahbari et al., 2016). Studies have also identified 'mutational signatures' that reflect age, mutagen exposures and DNA repair mechanisms (Alexandrov et al., 2013a, 2020; Rahbari et al., 2016). Although not as finely detailed in scale, one important study took advantage of herbarium samples to demonstrate that this type of de novo mutation occurred during colonization and expansion in the United States of a single lineage of *A. thaliana* (Exposito-Alonso et al., 2018). *Arabidopsis thaliana* is an annual selfing plant, which is therefore almost entirely homozygous across its diploid genome. Exposito-Alonso et al. (2018) discovered *de novo* mutations that were associated with genes related to adaptive traits that may have been selected during the establishment and expansion of this species.

Epimutations occur much more frequently than genetic mutations, they do not occur randomly across the genome, and they occur more often in genic regions than in transposable elements (reviewed in Richards et al., 2017). A recent whole genome survey of *Populus trichocarpa* showed epimutation rates that were very similar to *A. thaliana* on a per generation basis in the range of $10^{-3}$ to $10^{-4}$ (Hofmeister et al., 2020). Another study in maize showed that the forward epimutation rate was about 10 times larger than the backward epimutation rate, and two orders of magnitude larger than that of DNA mutation rate (Xu et al., 2020). In humans, the epimutation rate appears to be lower than in *A. thaliana* but was also estimated to be over two orders of magnitude greater than the germline genetic mutation rate (Carja et al., 2017). Unlike the extensive focus in cancer, how these mutation and epimutation rates translate into function has not been explored in invasive organisms, or in clonal plants more generally. Unfortunately, even with the most accurate sequencing platforms and assembly methods currently available, the technological challenges of accurately detecting mutation and epimutation indicate that this type of information is not yet within our reach for most non-model species (Yoder and Tiley, 2021).

## What We Know About Genomics of Cancer Metastasis

Even before the era of cancer genomics, extensive studies had revealed that "**genetic instability**" was a hallmark of cancer (Coffey, 1998; Duesberg et al., 1998). Mutations rates are higher in cells with genetic instability (Weisenberger et al., 2006; Hanahan and Weinberg, 2011; Loeb, 2011). Such is the case for cancer cells. The increased genetic variation that results from this mutation can result in phenotypic variation that has different fitness benefits for cells based on their ability to divide, migrate, and survive environmental conditions (Amend et al., 2016; Lloyd et al., 2016; Ibrahim-Hashim et al., 2017; Somarelli, 2021). The microenvironment of the tumor selects on this variation in phenotype and determines which cell lines will die, proliferate, or metastasize. Cancer cells that metastasize start in the selective environment of the primary tumor, then travel through lymphatic tissue or blood vessels to a distant organ (**Figure 1** and **Box 1: Figure A**). During this journey, metastatic cancer cells must evade immune cells and ultimately compete with healthy cells for resources when they reach a distal organ (Lloyd et al., 2016; Amend et al., 2016), all of which is mediated by genomic processes.

Cancer genomics was launched in 2006, but the sample information was limited in many cases (Ledford, 2010; Nature, 2020). Early analyses of cancer genomes showed that they carried thousands to tens of thousands of somatic mutations along with aneuploidies and genome doubling (˜30% of cases) (Stratton, 2011; Williams et al., 2019). While the vast majority of mutations were thought to have no biological function, they have been informative to understand the evolutionary history of cancers. Researchers have been able to develop algorithms that predict evolutionary fates of cell lineages based on population genetics concepts (Nik-Zainal et al., 2012; Williams et al., 2019). Nik-Zainal et al. (2012) identified a collection of somatic mutations shared by all cancer cells within a given breast cancer sample and used this concept to identify discrete clones and subclones. In order to do so, they examined the details of one patient (sequenced to 188× depth) and found that in the aneuploid tumor there were 70,690 somatic mutations genome-wide, many of which were in fewer than 5% of the reads for a given location in the genome. Their model predicted that 26,762 of these mutations (∼38%; including in genes TP53, PIK3CA, GATA3, MLL3, SMAD4, and NCOR1) along with trisomy 1q and several other rearrangements were found in every tumor cell indicating that some ancestral cell carried all of these somatic mutations. From this point, they could reconstruct the emergence of additional subclones as well [i.e., their subclone labeled cluster C represented 65% of the tumor cells, cluster B represented 18% of tumor cells, and cluster A accounted for 14% of tumor cells (Nik-Zainal et al., 2012)]. They concluded that large-scale chromosomal changes did not start to occur until after at least 15–20% of the point mutations had already occurred. Hence, instability at the chromosome level was not usually the earliest source of mutation in this breast cancer. Across the 20 breast cancer samples, they found a dominant subclonal lineage represented 50–95% of tumor cells, but a considerable proportion of somatic genetic variation was in only a fraction of tumor cells.

More recent studies of the PCAWG Consortium of the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas have provided further evidence of the power of cancer genomics to contribute to our understanding of shared and unique evolutionary genomic mechanisms (Alexandrov et al., 2020; Gerstung et al., 2020; ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium, 2020). Gerstung et al. (2020) used whole genome sequencing in cancer samples from 2,658 unique donors across 38 cancer types. They took advantage of the same approaches as Nik-Zainal et al. (2012) using sequence data to measure the number of copies of different alleles. They used this information to define categories of early and late clonal variants, the order in which variants arise, and the most recent common ancestor (MRCA) of all cancer cells in a tumor sample. Using phylogenetic reconstruction of mutations as a clock they mapped mutation timing estimates onto approximate real time to reconstruct the evolutionary

trajectories of cancer to even before the point of diagnosis. They presented timing and typical sequences of mutations as well as how drivers and mutational signatures varied across each cancer type.

Among the many interesting findings, Gerstung et al. (2020) identified an increase in diversity of mutated driver genes at later stages of tumor development, and 50% of all early clonal driver mutations occured in just nine genes (although see concerns raised by Plutynski, 2021). In many cases, the earliest events included TP53 mutations, as well as losses of chromosome 17 and most other highly recurrent cancer genes, such as KRAS, TERT, and CDKN2A. Whole genome duplication events occurred after tumors had accumulated several driver mutations, and many chromosomal gains and losses typically occurred later. This confirmed a long-held prediction in colorectal cancer called the "APC-KRAS-TP53 progression model" of Fearon and Vogelstein (1990). This finding concurs with several previous studies that had reported that very early events in cancer evolution occur in a few common drivers, and a more diverse array of drivers is involved in late tumor development (Jamal-Hanjani et al., 2017; Hu et al., 2019). Overall, the study showed that the spectrum of mutations changed throughout tumor evolution in 40% of samples. There were some common trends among tumors as they evolved, but they all followed diverse paths. Other studies demonstrated large differences in the underlying mutation rate among individual tumors and tumor types and emphasized that only a handful of mutations occur at appreciable frequencies across all cancer types; for example, only mutations in TP53 and PIK3CA occurred at a frequency of greater than 10% across cancer types in one study (Kandoth et al., 2013). Further, Alexandrov et al. (2013b, 2020) recently evaluated 84,729,690 somatic mutations from 4,645 whole-genomes and 19,184 exomes across most cancer types and made associations of signatures to exogenous or endogenous exposures, as well as to defective DNA-maintenance processes. They identified positive correlations between the age at cancer diagnosis and the number of mutations attributable to a signature, and that the underlying mutational process was active throughout the entire evolution of the lineage from normal cells.

The application of genomics to the study of metastases is of particular interest for understanding the genomics of invasions. The genetic and non-genetic alterations underlying cancer metastasis vary depending on the type of tumor as well as the stage of metastasis (Nguyen and Massagué, 2007). Genome-wide analysis of gene-expression in tumors has been applied to hematological cancers (Golub et al., 1999; Alizadeh et al., 2000), followed by solid tumors (Ramaswamy et al., 2003) to find signatures for predicting metastasis. Genes involved in initiation of metastasis promote invasion of the **basement membrane** and entry into the circulatory system. For example, loss of CASp8 (caspase 8) activation can protect cancer cells from apoptosis during invasion (Stupack et al., 2006). Epigenetic modifications can also initiate the metastatic state, particularly since mutations in epigenetic machinery can reshape the epigenome (Feinberg et al., 2006; Timp and Feinberg, 2013). Chromatin regulators are often mutated in cancer [e.g., mutations in the SWI/SNF complex occur in over 20% of all cancers (Kadoch and Crabtree, 2015)]. Further, a study in patients of glioblastoma multiforme evaluating SNP-genotypes, methylation, copy number variants, and gene expression data found that whole genome DNA methylation was the most informative molecular level predictor of survival (Bernal Rubio et al., 2018).

Recent studies indicated that even when specific genetic mutations instigated the invasion process, completion of the process depended only on non-genetic changes, specifically epigenetic changes that complement the genetic mutations (Lambert et al., 2017). Metastatic progression depends on the expression of genes that have specific functions as the cancer cell first becomes a CTC, then a DTC and finally an expanding micrometastasis. The expression of such genes during the metastatic cascade may provide quite different functions than they do for cancer cells of the primary tumor. Such genes include EREG that encodes COX2 and MMP1 (Gupta et al., 2007; Kuramochi et al., 2012; Qu et al., 2016). These remodel the vasculature in sites of metastasis and simultaneously, facilitate intravasation and angiogenesis at the primary tumor site (Gupta et al., 2007). Genes that are not involved in the primary tumor but facilitate metastasis at distal sites are classified as "metastasis-virulent". An example is CXCR4, a cytokine receptor that mediates cancer survival in a distant organ where its ligand CXCR12 is abundant in tissue microenvironments like bone marrow (Müller et al., 2001; Kang et al., 2003).

Metastatic samples can have higher mutation rates, specific types of mutations, aneuploidy or whole genome doubling and non-genetic effects compared to primary cancer cell counterparts (Alexandrov et al., 2013a,b, 2020; Martincorena et al., 2015). Chromosome instability also creates aneuploidy and promotes tumor evolution (Ben-David and Amon, 2020) and can result in dissimilarities between metastatic and primary tumors. However, a study of 118 biopsies of colorectal cancer with metastases to the liver or brain showed little divergence between the primary tumor and metastasis, and that "driver genes" were acquired early in the process of tumor progression. In fact, cells that "disseminated early" were more likely to seed metastases when the primary tumor was still clinically undetectable (Hu et al., 2019). Similar findings were reported in a study of two breast cancer patients: primary tumors and associated metastases were similar in gene expression and somatic mutation patterns (Hoadley et al., 2016). On the other hand, clones seeding metastasis in breast cancer in another study disseminated late from primary tumors and continued to acquire mutations. Further, distant metastases acquired driver mutations that were not seen in the primary tumor, including a wider repertoire of cancer genes than early drivers, e.g., inactivation of SWI-SNF and JAK2-STAT3 pathways (Yates et al., 2017).

For the purposes of understanding specific genomic response to invaded habitats, a particularly compelling study was recently completed on clear-cell renal cell carcinoma (ccRCC) tumors (Turajlic et al., 2018). Across 463 primary and 169 matched metastatic regions from 38 patients, Turajlic et al. (2018) found the number of driver events was lower in metastases (mean = 9), compared to primary tumors, and that metastases were significantly more homogeneous than primary tumors: 456 driver events were shared between primary tumors and

metastases, 230 were only found in primary tumors, and 39 driver events were only found in metastases. They further determined behavior of clonal lineages within the primary tumor by dividing 253 clones in the 38 patients into: (1) clones that were not represented in the metastatic samples ($n$ = 130 clones, defined as subclonal in the primary tumor and absent in metastasis), (2) clones that were maintained ($n$ = 38 clones, defined as the MRCA clones, clonal in both primary tumor and metastasis), and (3) clones that were selected ($n$ = 85 clones, defined as subclonal in the primary and clonal in metastasis; or absent in the primary and present in metastasis). Comparing selected versus unselected mutations, they found hallmark genomic alterations in ccRCC metastasis but also report the fascinating finding of specific and shared mutations associated with metastases across 18 different invaded tissues (Turajlic et al., 2018; **Box 1: Figure A**). This type of detailed information about genomic modifications associated with the invasion of "habitat types" is unprecedented in ecological studies and provides information not only about current status of populations but evolutionary history and therefore potential prevention. While each patient exhibited some unique features, this study also demonstrates the remarkable degree of parallel and convergent evolution in both the primary and metastatic tumors of different patients of this cancer type.

## THE ILLUSIVE UNIVERSAL PREDICTOR OF INVASIVE POTENTIAL-MANAGEMENT ISSUES

There are often intensive management responses to both invasive species and cancer, although the stakes differ in notable ways. Untreated, metastatic cancer is inevitably deadly (Wells et al., 2013), while the impacts of invasive species generally include economic costs ranging from minor to immense (Bradshaw et al., 2016; Diagne et al., 2021), and disruption of ecological communities, in some cases including native species extinction (Bellard et al., 2016a). Another striking difference between cancer and invasive species is general agreement that cancer is bad whereas some or even all stakeholders may see an invasive species neutrally or positively (e.g., burros in the Grand Canyon, stocking non-native game species, and non-native biological control agents). Regardless, several of the similarities and differences in management approaches could be addressed by a better understanding of the genomic mechanisms in context.

It is widely argued that the best way to reduce ecological and economic costs of invasive species is to interfere with the transport and establishment steps of invasion (Keller et al., 2007; Bailey et al., 2011). A primary reason is that once species have become invasive in a new habitat, eradication is rarely feasible, except on some islands (Parkes and Panetta, 2009; Moon et al., 2015). Damage or costs related to damage reduction become recurring (Liebhold et al., 2016). Cancer treatment is similar with regards to the importance of cancer prevention and early detection. Ecologists use species traits or ecological niche models to identify species of concern and locations of high risk, whereas doctors can use individual traits, such as environmental exposures, lifestyle, age, or genetic predispositions to guide cancer surveillance (Dobson, 2013; Katzke et al., 2015; Bernal Rubio et al., 2018; Hu et al., 2021). But this often applies to preventing cancer initiation in the first place. With respect to preventing metastases, there is growing interest in therapies that target CTCs (Ortiz-Otero et al., 2020) as well as bolstering normal tissues and the immune system to prevent the establishment of DTCs (Risson et al., 2020).

Surveillance of invasive species aimed at preventing introduction of new propagules often lapses after a species is established. While understandable, this may be unwise. Further import of new individuals into an already invaded habitat likely provides additional heritable variation (e.g., Kolbe et al., 2004), potentially allowing for faster adaptation to new habitats or increased success in the invaded range. In cancer treatment, the focus on removing as many of the cancer cells as possible has the effect of reducing the variation present, potentially pushing cancer back toward earlier, less invasive stages, which seems to be effective for about 50% of the cases (Pienta et al., 2020a). However, this approach also selects for those few cells that are able to resist therapy and may require a different management strategy (Ibrahim-Hashim et al., 2017; Gatenby and Brown, 2020; Pressley et al., 2021). This line of reasoning suggests increased focus on preventing introductions may still be appropriate and cost-effective for existing invaders. Similarly identifying the molecular features associated with progression to invasions is a major objective in cancer research (Srivastava et al., 2018). The potential for this type of biomarker approach was recently highlighted in a study of colorectal cancer with metastases to the liver or brain. The early mutations in "driver genes" were associated with seeding metastases. These key mutations were in an independent cohort of 2,751 colorectal cancers (Hu et al., 2019) and could be the targets of therapy, enhancing a personalized medicine approach. Analysis of multi-omics data and development of new statistics approaches that can integrate these data will be an imperative to identify the relative contributions of different molecular level mechanisms that underlie cancer progression, invasion and response to environmental challenges more generally (Bernal Rubio et al., 2018; Hofmeister et al., 2020; Nam et al., 2021; Teschendorff and Feinberg, 2021; Yoder and Tiley, 2021).

Once invasion has taken place the management regime diverges between cancer treatment and invasive species management. Invasive species management tends to focus on slowing the spread (Sharov et al., 2002) or protecting specific habitats (e.g., Short et al., 1992), or even tolerance (Schlaepfer et al., 2011) and resignation (Regulations.gov, 2020). Cancer treatment initially tends to take a much more aggressive treatment approach, often combining several methods with the goal of eradication and long term suppression of tumors (Blagosklonny, 2004; Yap et al., 2013; Gatenby and Brown, 2020). These treatments are often expensive and have intense side effects, but genomic analysis of tumors may provide directly relevant information for selecting treatments most likely to be effective (Bozic et al., 2012).

When detected and treated early enough, most cancers are curable. Cure generally involves surgical resection of the tumor and/or radiation therapy. So long as the resected or irradiated tumor contains all of the cancer cells, then knowledge of

the genomics becomes less relevant. However, identifying the whereabouts of all of the cancer cells involves some guesswork. Hence, neoadjuvant drug therapies prior to surgery help ensure a contained population of cancer cells, and adjuvant drug therapies after surgery aim to eliminate undetectable surviving fragments of the primary tumor or micrometastases elsewhere in the body. Both neoadjuvant and adjuvant therapies can be improved based on genetic and molecular markers of the cancer cells' state and heterogeneity (Dressman et al., 2006; Duran et al., 2020; Oshi et al., 2020). Upon detection or at the start of a management program, complete elimination of an invasive species often succeeds or fails based on the ability to find and cull all individuals. For both cancer and invasive species, if the management regime does not kill them all, the cancer and pest species will evolve resistance (Pressley et al., 2021). In cancer, understanding the genomics of resistance promises insight to why initial therapies do not or cannot cure the patient.

Despite the limitations for understanding the translation of genome level processes into traits in most species, genomics is becoming part of the invasive species management toolbox because these approaches can provide accurate diagnostics of invasive species (Cristescu, 2015; Hamelin and Roe, 2020). We are unaware of any invasive species management regimes that have been truly shaped by genomic knowledge (see Stewart et al., 2009), but molecular analyses can provide taxonomic clarification, evidence of hybridization and cryptic species, population structure and origin of invasions for management purposes (Gaskin et al., 2011; Chown et al., 2015). Studies on knotweed for instance, have shown that closely related taxa had dramatically different responses to herbicide application and that the hybrid *Reynoutria x. bohemica* is particularly resilient (Bímová et al., 2001). The genomic mechanisms underlying these differences have not yet been examined. Genomics approaches are now being used to identify appropriate biocontrol agents (Sun et al., 2020; van Steenderen et al., 2021; Harms et al., 2021). For example, specific cochineal insects in the genus *Dactylopius* (Hemiptera: Dactylopiidae) are effective biocontrol agents of some invasive *Opuntia* cactus species. But the different *Dactylopius* species are so morphologically similar that numerous misidentifications have contributed to failed attempts at biological control. van Steenderen et al. (2021) report that nucleotide sequencing of three gene regions (12S rRNA, 18S rRNA, and COI) and two inter-simple sequence repeats (ISSR) were effective in identifying the target species *D*actylopius *opuntiae* and *Dactylopius tomentosus* and even different lineages within *D. tomentosus*. A study of invasive *Ambrosia artemisiifolia* found that the genotype of the leaf beetle *Ophraella communa* determined potential success as a biocontrol agent, but the specific genomic mechanisms of that association were not investigated (Sun et al., 2020).

## CONCLUSION

In the last 50 years, foundational concepts in ecological and evolutionary genetics have been applied to both the study of invasive species and the study of cancers. We have discussed many similarities in the application of genomics to cancer and invasive species (summarized in **Table 2** with reference to questions outlined recently by Dujon et al., 2021 for cancer and Bock et al., 2015 and van Kleunen et al., 2018 for ecological invasions). Cancer cells and invasive species alter their environment and can cause extinctions of other cells or organisms because they alter the composition of their habitat or deplete resources. Ecological studies have a stronger history of describing this process across a diversity of species and habitat interactions, but the molecular mechanisms underlying this could be informed by genomics, as we have seen in cancer studies (e.g., **Box 1: Figure A**; Turajlic et al., 2018).

There are some important outstanding questions in invasive species ecology that do not easily find parallels in cancer studies. For example: van Kleunen et al. (2018) highlighted the importance of questions like "What will be the future global distribution of alien plants?," "How important are mutualists compared with antagonists in driving invasions?" and "How frequent is rapid coevolution of aliens and natives?" which have some parallels in cancer but do not have obvious analogs in Dujon et al. (2021; **Table 2A**). These include, for instance, the cooperative interactions that might occur between DTCs, or how clusters of CTCs may increase likelihoods of metastases (Fabisiewicz and Grzybowska, 2017). This aligns with invasions in nature where success generally increases with the number of individuals introduced simultaneously to the novel location (Barney and Whitlow, 2008).

Bock et al. (2015) highlight important questions that can be specifically addressed with genomics approaches in invasive species studies like "why hybridization sometimes results in increased colonization success and sometimes does not," "whether the accumulation of deleterious mutations limits invasions and/or if compensatory mechanisms reduce the severity of expansion load," and "the extent of gene re-use during the evolution of invaders" which are not particularly relevant in cancers. However, both van Kleunen et al. (2018) and Bock et al. (2015) emphasize the importance of the outstanding question: "What explains the existence and length of lag phases?," which is also unknown in cancers (e.g., "What molecular level processes differentiate benign versus malignant tumors?"; **Table 2B**) and has great potential for therapeutic targets. Other major common themes highlighted in the table address questions about the molecular level mechanisms involved in initiation and progression, the importance of plasticity at various stages, the importance of habitat suitability and our ability to use genomics in predictive modeling. Genomics approaches promise to inform our understanding about these outstanding questions in both cancer and ecological studies.

We have argued that the detailed studies of the behavior of a variety of human cancers can inform our understanding of genome level dynamics in the diversity of invasive species and provide predictive frameworks for management. However, despite the tremendous efforts of the last 15 years, the transitions from normal to cancerous conditions or from primary tumor to metastasis (Turajlic et al., 2018; Hu et al., 2019) are still not well understood. Even normal cells contain many mutations that accrue with age, and some genic regions have a higher mutation

rate than others (Martincorena and Campbell, 2015; Martincorena et al., 2015, 2018; Gao et al., 2019; Goldmann et al., 2019; Zahir et al., 2020). It is particularly challenging that there is no discrete boundary between normal ageing processes and cancer evolution (Lee-Six et al., 2018, 2019; Moore et al., 2020). In addition, changes in methylation more strongly predicted survival in patients with glioblastoma multiforme than genetic polymorphisms and methylation was strongly associated with age (Bernal Rubio et al., 2018). Further, the microenvironment of the pre-tumor is also aging and this could contribute to tumorigenesis and subsequent progression (Zahir et al., 2020).

As with the pre-invasive stage of invasive species in ecology (Vandepitte et al., 2014), premalignancy in solid tumors has not been well studied, partly because of the challenge of early detection (Gerstung et al., 2020; Zahir et al., 2020). Several researchers have concluded that a comprehensive understanding of the progression of cancer requires understanding not only at the molecular level but also at the phenotypic and ecological level such as physiological, structural, and environmental information that occurs spatially and temporally (Ibrahim-Hashim et al., 2017, 2021; Zahir et al., 2020; Nam et al., 2021). While this is also potentially the holy grail for understanding the progression of invasive species, in the case of cancer, the stakes are higher and very immediate to the patient. Hence, Zahir et al. (2020) have reviewed how sophisticated techniques have been developed for multiplexing genomic, proteomic and transcriptomic analysis in situ, while preserving the spatial relationships between cells within their native tissue architecture and immune context. These include finely dissected spatial transcriptomic profiling and single-molecule fluorescence in situ hybridization (smFISH), where transcripts are directly labeled in tissue sections to image and visualize their subcellular locations. Cancer studies can also include manipulations to verify functional relationships thanks to the Cancer Cell Line Encyclopedia (CCLE), application of CRISPR loss-of-function methods, cell-viability data for thousands of compounds which define a "cancer dependency map," and single cell sequencing technologies (Tsherniak et al., 2017; Williams et al., 2019; Teschendorff and Feinberg, 2021).

While this outstanding level of resources may never be available in any invasive species, the enormous amount of data that is already accumulated and will continue to accumulate could be combined with the nuanced, and crafty (out of necessity) approaches of evolutionary ecologists to provide a better understanding of the translation of genotype to phenotype. Doing so is likely to improve risk analysis and management interventions.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

This work was supported by the Federal Ministry of Education and Research (BMBF; MOPGA Project ID 306055 to CR), NIH/NCI 1U54CA193489-01A1, Cancer as a Complex Adaptive System (to JB and AI-H), and NIH/NCI U54 Supplement, The tumor-host evolutionary arms race (to JB, AI-H, and CR).

## ACKNOWLEDGMENTS

We thank the editors for the opportunity to pursue these ideas. We acknowledge support by the Open Access Publishing Fund of University of Tübingen.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.681100/full#supplementary-material

## REFERENCES

Aceto, N., Bardia, A., Miyamoto, D. T., Donaldson, M. C., Wittner, B. S., and Spencer, J. A. (2014). Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* 158, 1110–1122. doi: 10.1016/j.cell.2014.07.013

Aikio, S., Duncan, R. P., and Hulme, P. E. (2010). Lag-phases in alien plant invasions: separating the facts from the artefacts. *Oikos* 119, 370–378. doi: 10.1111/j.1600-0706.2009.17963.x

Alexandrov, L. B., Australian Pancreatic, Cancer Genome, Initiative, Nik-Zainal, S., Wedge, D. C., et al. (2013a). Signatures of mutational processes in human cancer. *Nature* 500, 415–421.

Alexandrov, L. B., Kim, J., Haradhvala, N. J., Huang, M. N., Tian, Ng, A. W., et al. (2020). The repertoire of mutational signatures in human cancer. *Nature* 578, 94–101.

Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Campbell, P. J., and Stratton, M. R. (2013b). Deciphering signatures of mutational processes operative in human cancer. *Cell Rep.* 3, 246–259. doi: 10.1016/j.celrep.2012.12.008

Alix-Panabières, C., and Pantel, K. (2021). Liquid biopsy: from discovery to clinical application. *Cancer Discov.* 11, 858–873. doi: 10.1158/2159-8290.cd-20-1311

Alizadeh, A. A., Eisen, M. B., Davis, R. E., Ma, C., Lossos, I. S., and Rosenwald, A. (2000). Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403, 503–511.

Allendorf, F. W., and Lundquist, L. L. (2003). Introduction: population biology, evolution, and control of invasive species. *Conserv. Biol. J. Soc. Conserv. Biol.* 17, 24–30. doi: 10.1046/j.1523-1739.2003.02365.x

Alvarez, M., Schrey, A. W., and Richards, C. L. (2015). Ten years of transcriptomics in wild populations: what have we learned about their ecology and evolution? *Mol. Ecol.* 24, 710–725. doi: 10.1111/mec.13055

Amend, S. R., Roy, S., Brown, J. S., and Pienta, K. J. (2016). Ecological paradigms to understand the dynamics of metastasis. *Cancer Lett.* 380, 237–242. doi: 10.1016/j.canlet.2015.10.005

Ardura, A., Zaiko, A., Morán, P., Planes, S., and Garcia-Vazquez, E. (2017). Epigenetic signatures of invasive status in populations of marine invertebrates. *Sci. Rep.* 7:42193.

Arnold, P. A., Nicotra, A. B., and Kruuk, L. E. B. (2019). Sparse evidence for selection on phenotypic plasticity in response to temperature. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 374:20180185. doi: 10.1098/rstb.2018.0185

Bailey, J. P., and Conolly, A. P. (2000). Prize-winners to pariahs - a history of Japanese knotweed s.l. (Polygonaceae) in the British Isles. *Watsonia* 23, 93–110.

Bailey, J. P., Bímová, K., and Mandák, B. (2009). Asexual spread versus sexual reproduction and evolution in Japanese Knotweed s.l. sets the stage for the "Battle of the Clones. *Biol. Invasions* 11, 1189–1203. doi: 10.1007/s10530-008-9381-4

Bailey, S. A., Deneau, M. G., Jean, L., Wiley, C. J., Leung, B., and MacIsaac, H. J. (2011). Evaluating efficacy of an environmental policy to prevent biological invasions. *Environ. Sci. Technol.* 45, 2554–2561. doi: 10.1021/es102655j

Baker, H. G., and Stebbins, G. L. (1965). *The Genetics of Colonizing Species*. New York: Academic Press.

Banta, J. A., and Richards, C. L. (2018). Quantitative epigenetics and evolution. *Heredity* 121, 210–224 doi: 10.1038/s41437-018-0114-x

Barney, J. N., and Whitlow, T. H. (2008). A unifying framework for biological invasions: the state factor model. *Biol. Invasions* 10, 259–272. doi: 10.1007/s10530-007-9127-8

Barriere, G., Fici, P., Gallerani, G., Fabbri, F., and Rigaud, M. (2015). Epithelial mesenchymal transition: a double-edged sword. *Clin. Transl. Med.* 4:14.

Bartz, R., and Kowarik, I. (2019). Assessing the environmental impacts of invasive alien plants: a review of assessment approaches. *NeoBiota* 43, 69–99. doi: 10.3897/neobiota.43.30122

Becker, C., Hagmann, J., Müller, J., Koenig, D., Stegle, O., Borgwardt, K., et al. (2011). Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature* 480, 245–249. doi: 10.1038/nature10555

Beerling, D. J., Bailey, J. P., and Conolly, A. P. (1994). Fallopia Japonica (Houtt.) Ronse Decraene. *J. Ecol.* 82:959. doi: 10.2307/2261459

Bellard, C., Cassey, P., and Blackburn, T. M. (2016a). Alien species as a driver of recent extinctions. *Biol. Lett.* 12:20150623. doi: 10.1098/rsbl.2015.0623

Bellard, C., Genovesi, P., and Jeschke, J. M. (2016b). Global patterns in threats to vertebrates by biological invasions. *Proc. Biol. Sci.* 283:20152454. doi: 10.1098/rspb.2015.2454

Ben-David, U., and Amon, A. (2020). Context is everything: aneuploidy in cancer. *Nat. Rev. Genet.* 21, 44–62. doi: 10.1038/s41576-019-0171-x

Bernal Rubio, Y. L., González-Reymúndez, A., Wu, K.-H. H., Griguer, C. E., Steibel, J. P., and de Los Campos, G. (2018). Whole-genome multi-omic study of survival in patients with glioblastoma multiforme. *G3* 8, 3627–3636. doi: 10.1534/g3.118.200391

Bímová, K., Mandák, B., and Pyšek, P. (2001). "Experimental control of Reynoutria congeners: a comparative study of a hybrid and its parents," in *Invasions: Species Ecology and Ecosystem Management*, eds G. Brundu, J. Brock, I. Camarda, L. Child, Wade, and MPlant (Leiden: Backhuys Publishers).

Birkbak, N. J., and McGranahan, N. (2020). Cancer genome evolutionary trajectories in metastasis. *Cancer Cell* 37, 8–19. doi: 10.1016/j.ccell.2019.12.004

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., et al. (2011). A proposed unified framework for biological invasions. *Trends Ecol. Evol.* 26, 333–339. doi: 10.1016/j.tree.2011.03.023

Blagosklonny, M. V. (2004). Analysis of FDA approved anticancer drugs reveals the future of cancer therapy. *Cell Cycle* 3, 1035–1042.

Blossey, B., and Notzold, R. (1995). Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *J. Ecol.* 83, 887–889. doi: 10.2307/2261425

Bock, D. G., Caseys, C., Cousens, R. D., Hahn, M. A., Heredia, S. M., Hübner, S., et al. (2015). What we still don't know about invasion genetics. *Mol. Ecol.* 24, 2277–2297.

Bock, D. G., Kantar, M. B., Caseys, C., Matthey-Doret, R., and Rieseberg, L. H. (2018). Evolution of invasiveness by genetic accommodation. *Nat. Ecol. Evol.* 2, 991–999. doi: 10.1038/s41559-018-0553-z

Bodt, L. H., Rollins, L. A., and Zichello, J. M. (2020). Contrasting mitochondrial diversity of European starlings (*Sturnus vulgaris*) across three invasive continental distributions. *Ecol. Evol.* 10, 10186–10195. doi: 10.1002/ece3.6679

Bonduriansky, R., and Day, T. (2018). *Extended Heredity*. Princeton, NJ: Princeton University Press.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., and Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144, 1–11. doi: 10.1007/s00442-005-0070-z

Bozic, I., Allen, B., and Nowak, M. A. (2012). Dynamics of targeted cancer therapy. *Trends Mol. Med.* 18, 311–316.

Bradshaw, C. J. A., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., et al. (2016). Massive yet grossly underestimated global costs of invasive insects. *Nat. Commun.* 7:12986.

Britton, J. R., and Gozlan, R. E. (2013). How many founders for a biological invasion? predicting introduction outcomes from propagule pressure. *Ecology* 94, 2558–2566. doi: 10.1890/13-0527.1

Cairns, J. (1975). Mutation selection and the natural history of cancer. *Nature* 255, 197–200. doi: 10.1038/255197a0

Capp, J.-P., and Thomas, F. (2020). A similar speciation process relying on cellular stochasticity in microbial and cancer cell populations. *iScience* 23:101531. doi: 10.1016/j.isci.2020.101531

Card, D. C., Perry, B. W., Adams, R. H., Schield, D. R., Young, A. S., and Andrew, A. (2018). Novel ecological and climatic conditions drive rapid adaptation in invasive Florida Burmese pythons. *Mol. Ecol.* 27, 4744–4757. doi: 10.1111/mec.14885

Carja, O., MacIsaac, J. L., Mah, S. M., Henn, B. M., Kobor, M. S., Feldman, M. W., et al. (2017). Worldwide patterns of human epigenetic variation. *Nat. Ecol. Evol.* 1, 1577–1583. doi: 10.1038/s41559-017-0299-z

Castoe, T. A., de Koning, A. P. J., Hall, K. T., Card, D. C., Schield, D. R., Fujita, M. K., et al. (2013). The Burmese python genome reveals the molecular basis for extreme adaptation in snakes. *Proc. Natl. Acad. Sci. USA* 110, 20645–20650. doi: 10.1073/pnas.1314475110

Castro-Giner, F., and Aceto, N. (2020). Tracking cancer progression: from circulating tumor cells to metastasis. *Genome Med.* 12:31.

Catford, J. A., Jansson, R., and Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Divers. Distrib.* 15, 22–40. doi: 10.1111/j.1472-4642.2008.00521.x

Chen, C., Zheng, Z., Bao, Y., Zhang, H., Richards, C. L., Li, J., et al. (2020). Comparisons of natural and cultivated populations of *Corydalis yanhusuo* indicate divergent patterns of genetic and epigenetic variation. *Front. Plant Sci.* 11:985.

Chen, K.-W., and Pienta, K. J. (2011). Modeling invasion of metastasizing cancer cells to bone marrow utilizing ecological principles. *Theor. Biol. Med. Model.* 8:36.

Chen, Y., Zhao, L., Teng, H., Shi, C., Liu, Q., Zhang, J., et al. (2021). Population genomics reveal rapid genetic differentiation in a recently invasive population of *Rattus norvegicus*. *Front. Zool.* 18:6.

Chown, S. L., Hodgins, K. A., Griffin, P. C., Oakeshott, J. G., Byrne, M., and Hoffmann, A. A. (2015). Biological invasions, climate change and genomics. *Evol. Appl.* 8, 23–46. doi: 10.1111/eva.12234

Coffey, D. S. (1998). Self-organization, complexity and chaos: the new biology for medicine. *Nat. Med.* 4, 882–885. doi: 10.1038/nm0898-882

Colautti, R. I., Alexander, J. M., Dlugosch, K. M., Keller, S. R., and Sultan, S. E. (2017). Invasions and extinctions through the looking glass of evolutionary ecology. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 372:1712.

Colautti, R. I., and Lau, J. A. (2015). Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Mol. Ecol.* 24, 1999–2017. doi: 10.1111/mec.13162

Cristescu, M. E. (2015). Genetic reconstructions of invasion history. *Mol. Ecol.* 24, 2212–2225. doi: 10.1111/mec.13117

Cuthbertab, R. N., Pattison, Z., Taylor, N. G., Verbrugge, L., Diagne, C., Ahmed, D. A., et al. (2021). Global economic costs of aquatic invasive alien species. *Sci. Total Environ.* 775:145238. doi: 10.1016/j.scitotenv.2021.145238

Davidson, A. M., Jennions, M., and Nicotra, A. B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis: Invasive species have higher phenotypic plasticity. *Ecol. Lett.* 14, 419–431. doi: 10.1111/j.1461-0248.2011.01596.x

de Groot, A. E., Roy, S., Brown, J. S., Pienta, K. J., and Amend, S. R. (2017). Revisiting seed and soil: examining the primary tumor and cancer cell foraging in metastasis. *Mol. Cancer Res.* 15, 361–370. doi: 10.1158/1541-7786.mcr-16-0436

Del Tredici, P. (2017). The introduction of Japanese knotweed, *Reynoutria japonica*, into North America. *J. Torrey Bot. Soc.* 144, 406–416. doi: 10.3159/torrey-d-17-00002.1

Diagne, C., Leroy, B., Gozlan, R. E., Vaissière, A.-C., Assailly, C., Nuninger, L., et al. (2020). InvaCost, a public database of the economic costs of biological invasions worldwide. *Sci. Data* 7:277.

Diagne, C., Leroy, B., Vaissière, A.-C., Gozlan, R. E., Roiz, D., Jarić, I., et al. (2021). High and rising economic costs of biological invasions worldwide. *Nature* 592, 571–576. doi: 10.1038/s41586-021-03405-6

Diez, J. M., Sullivan, J. J., Hulme, P. E., Edwards, G., and Duncan, R. P. (2008). Darwin's naturalization conundrum: dissecting taxonomic patterns of species invasions. *Ecol. Lett.* 11, 674–681. doi: 10.1111/j.1461-0248.2008.01178.x

Dlugosch, K. M., and Parker, I. M. (2008a). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* 17, 431–449. doi: 10.1111/j.1365-294x.2007.03538.x

Dlugosch, K. M., and Parker, I. M. (2008b). Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecol. Lett.* 11, 701–709. doi: 10.1111/j.1461-0248.2008.01181.x

Dlugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A., and Gillette, H. D. (2015). The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Mol. Ecol.* 24, 2095–2111. doi: 10.1111/mec.13183

Dobson, J. M. (2013). Breed-predispositions to cancer in pedigree dogs. *ISRN Vet. Sci.* 2013:941275.

Douhovnikoff, V., and Dodd, R. S. (2015). Epigenetics: a potential mechanism for clonal plant success. *Plant Ecol.* 216, 227–233. doi: 10.1007/s11258-014-0430-z

Doyle, J. J., Flagel, L. E., Paterson, A. H., Rapp, R. A., Soltis, D. E., Soltis, P. S., et al. (2008). Evolutionary genetics of genome merger and doubling in plants. *Annu. Rev. Genet.* 42, 443–461. doi: 10.1146/annurev.genet.42.110807.091524

Dressman, H. K., Hans, C., Bild, A., Olson, J. A., Rosen, E., Marcom, P. K., et al. (2006). Gene expression profiles of multiple breast cancer phenotypes and response to neoadjuvant chemotherapy. *Clin. Cancer Res.* 12(3 Pt 1), 819–826. doi: 10.1158/1078-0432.ccr-05-1447

Dubin, M. J., Zhang, P., Meng, D., Remigereau, M.-S., Osborne, E. J., Paolo Casale, F., et al. (2015). DNA methylation in Arabidopsis has a genetic basis and shows evidence of local adaptation. *eLife* 4:e05255.

Duesberg, P., Rausch, C., Rasnick, D., and Hehlmann, R. (1998). Genetic instability of cancer cells is proportional to their degree of aneuploidy. *Proc. Natl. Acad. Sci. USA* 95, 13692–13697. doi: 10.1073/pnas.95.23.13692

Dujon, A. M., Aktipis, A., Alix-Panabières, C., Amend, S. R., Boddy, A. M., Brown, J. S., et al. (2021). Identifying key questions in the ecology and evolution of cancer. *Evol. Appl.* 14, 877–892.

Duran, G., Cruz, R., Simoes, A. R., Barros, F., Giráldez, J. M., Bernárdez, B., et al. (2020). Efficacy and toxicity of adjuvant chemotherapy on colorectal cancer patients: how much influence from the genetics? *J. Chemother.* 32, 310–322. doi: 10.1080/1120009x.2020.1764281

Eckert, S., Herden, J., Stift, M., Joshi, J., and van Kleunen, M. (2021). Manipulation of cytosine methylation does not remove latitudinal clines in two invasive goldenrod species in Central Europe. *Mol. Ecol.* 30, 222–236. doi: 10.1111/mec.15722

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., and Facon, B. (2016). Is there a genetic paradox of biological invasion? *Annu. Rev. Ecol. Evol. Syst.* 47, 51–72.

Exposito-Alonso, M., Becker, C., Schuenemann, V. J., Reiter, E., Setzer, C., Slovak, R., et al. (2018). The rate and potential relevance of new mutations in a colonizing plant lineage. *PLoS Genet.* 14:e1007155. doi: 10.1371/journal.pgen.1007155

Fabisiewicz, A., and Grzybowska, E. (2017). CTC clusters in cancer progression and metastasis. *Med. Oncol.* 34:12.

Fearon, E. R., and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* 61, 759–767. doi: 10.1016/0092-8674(90)90186-i

Feinberg, A. P., and Irizarry, R. A. (2010). Evolution in health and medicine Sackler colloquium: stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. *Proc. Natl. Acad. Sci. USA* 107(Suppl. 1), 1757–1764. doi: 10.1073/pnas.0906183107

Feinberg, A. P., Koldobskiy, M. A., and Göndör, A. (2016). Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. *Nat. Rev. Genet.* 17, 284–299. doi: 10.1038/nrg.2016.13

Feinberg, A. P., Ohlsson, R., and Henikoff, S. (2006). The epigenetic progenitor origin of human cancer. *Nat. Rev. Genet.* 7, 21–33. doi: 10.1038/nrg1748

Felden, A., Paris, C., Chapple, D. G., Suarez, A. V., Tsutsui, N. D., Lester, P. J., et al. (2019). Native and introduced Argentine ant populations are characterised by distinct transcriptomic signatures associated with behaviour and immunity. *Working Paper Series* 49, 105–126. doi: 10.3897/neobiota.49.36086

Fitzpatrick, B. M., Johnson, J. R., Kump, D. K., Shaffer, H. B., Smith, J. J., and Voss, S. R. (2009). Rapid fixation of non-native alleles revealed by genome-wide SNP analysis of hybrid tiger salamanders. *BMC Evol. Biol.* 9:176. doi: 10.1186/1471-2148-9-176

Flanagan, B. A., Krueger-Hadfield, S. A., Murren, C. J., Nice, C. C., Strand, A. E., and Sotka, E. E. (2021). Founder effects shape linkage disequilibrium and genomic diversity of a partially clonal invader. *Mol. Ecol.* 30, 1962–1978. doi: 10.1111/mec.15854

Flucher, S. M., Krapf, P., Arthofer, W., Suarez, A. V., Crozier, R. H., Steiner, F. M., et al. (2021). Effect of social structure and introduction history on genetic diversity and differentiation. *Mol. Ecol.* 30, 2511–2527. doi: 10.1111/mec.15911

Gao, L., Geng, Y., Li, B., Chen, J., and Yang, J. (2010). Genome-wide DNA methylation alterations of *Alternanthera philoxeroides* in natural and manipulated habitats: implications for epigenetic regulation of rapid responses to environmental fluctuation and phenotypic variation. *Plant Cell Environ.* 33, 1820–1827. doi: 10.1111/j.1365-3040.2010.02186.x

Gao, Z., Moorjani, P., Sasani, T. A., Pedersen, B. S., Quinlan, A. R., Jorde, L. B., et al. (2019). Overlooked roles of DNA damage and maternal age in generating human germline mutations. *Proc. Natl. Acad. Sci. USA* 116, 9491–9500. doi: 10.1073/pnas.1901259116

Gaskin, J. F., Bon, M.-C., Cock, M. J. W., Cristofaro, M., Biase, A. D., De Clerck-Floate, R., et al. (2011). Applying molecular-based approaches to classical biological control of weeds. *Biol. Control Theory Appl. Pest Manag.* 58, 1–21. doi: 10.1016/j.biocontrol.2011.03.015

Gatenby, R. A., and Brown, J. (2017). Mutations, evolution and the central role of a self-defined fitness function in the initiation and progression of cancer. *Biochim. Biophys. Acta Rev. Cancer* 1867, 162–166. doi: 10.1016/j.bbcan.2017.03.005

Gatenby, R. A., and Brown, J. S. (2020). The evolution and ecology of resistance in cancer therapy. *Cold Spring Harbor Perspect. Med.* 8:a033415. doi: 10.1101/cshperspect.a033415

Gatenby, R. A., Brown, J., and Vincent, T. (2009). Lessons from applied ecology: cancer control using an evolutionary double bind. *Cancer Res.* 69, 7499–7502. doi: 10.1158/0008-5472.can-09-1354

Geng, Y.-P., Pan, X.-Y., Xu, C.-Y., Zhang, W.-J., Li, B., Chen, J.-K., et al. (2007). Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. *Biol. Invasions* 9, 245–256. doi: 10.1007/s10530-006-9029-1

Gerber, E., Krebs, C., Murrell, C., Moretti, M., Rocklin, R., and Schaffner, U. (2008). Exotic invasive knotweeds (Fallopia spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. *Biol. Conserv.* 141, 646–654. doi: 10.1016/j.biocon.2007.12.009

Gerstung, M., Jolly, C., Leshchiner, I., Dentro, S. C., Gonzalez, S., Rosebrock, D., et al. (2020). The evolutionary history of 2,658 cancers. *Nature* 578, 122–128.

Goldmann, J. M., Veltman, J. A., and Gilissen, C. (2019). De novo mutations reflect development and aging of the human Germline. *Trends Genet.* 35, 828–839. doi: 10.1016/j.tig.2019.08.005

Golub, T. R., Slonim, D. K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J. P., et al. (1999). Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 286, 531–537. doi: 10.1126/science.286.5439.531

Grimsby, J. L., Tsirelson, D., Gammon, M. A., and Kesseli, R. (2007). Genetic diversity and clonal vs. sexual reproduction in Fallopia spp. (Polygonaceae). *Am. J. Bot.* 94, 957–964. doi: 10.3732/ajb.94.6.957

Gupta, G. P., Nguyen, D. X., Chiang, A. C., Bos, P. D., Kim, J. Y., Nadal, C., et al. (2007). Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature* 446, 765–770. doi: 10.1038/nature05760

Gurevitch, J., Fox, G. A., Wardle, G. M., Inderjit, K., and Taub, D. (2011). Emergent insights from the synthesis of conceptual frameworks for biological invasions. *Ecol. Lett.* 14, 407–418. doi: 10.1111/j.1461-0248.2011.01594.x

Hamelin, R. C., and Roe, A. D. (2020). Genomic biosurveillance of forest invasive alien enemies: a story written in code. *Evol. Appl.* 13, 95–115. doi: 10.1111/eva.12853

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Hapach, L. A., Mosier, J. A., Wang, W., and Reinhart-King, C. A. (2019). Engineered models to parse apart the metastatic cascade. *NPJ Precis. Oncol.* 3:20.

Harms, N. E., Williams, D. A., and Purcell, M. F. (2021). The role of overseas genetic surveys to potentially accelerate biological control development for a new *Hydrilla verticillata* introduction in the USA. *Biocontrol* 66, 271–280. doi: 10.1007/s10526-020-10050-x

Hawes, N. A., Fidler, A. E., Tremblay, L. A., Pochon, X., Dunphy, B. J., and Smith, K. F. (2018). Understanding the role of DNA methylation in successful biological invasions: a review. *Biol. Invasions* 20, 2285–2300. doi: 10.1007/s10530-018-1703-6

Hawkins, N. J., Bass, C., Dixon, A., and Neve, P. (2019). The evolutionary origins of pesticide resistance. *Biol. Rev. Camb. Philos. Soc.* 94, 135–155. doi: 10.1111/brv.12440

He, F., Steige, K. A., Kovacova, V., Göbel, U., Bouzid, M., Keightley, P. D., et al. (2021). Cis-regulatory evolution spotlights species differences in the adaptive potential of gene expression plasticity. *Nat. Commun.* 12:3376.

Herman, J. J., and Sultan, S. E. (2011). Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Front. Plant Sci.* 2:102.

Herman, J. J., and Sultan, S. E. (2016). DNA methylation mediates genetic variation for adaptive transgenerational plasticity. *Proc. R. Soc. B Biol. Sci.* 283:20160988. doi: 10.1098/rspb.2016.0988

Hoadley, K. A., Siegel, M. B., Kanchi, K. L., Miller, C. A., Ding, L., Zhao, W., et al. (2016). Tumor evolution in two patients with basal-like breast cancer: a retrospective genomics study of multiple metastases. *PLoS Med.* 13:e1002174. doi: 10.1371/journal.pmed.1002174

Hodgins, K. A., Bock, D. G., Hahn, M. A., Heredia, S. M., Turner, K. G., and Rieseberg, L. H. (2015). Comparative genomics in the Asteraceae reveals little evidence for parallel evolutionary change in invasive taxa. *Mol. Ecol.* 24, 2226–2240. doi: 10.1111/mec.13026

Hodgins, K. A., Lai, Z., Nurkowski, K., Huang, J., and Rieseberg, L. H. (2013). The molecular basis of invasiveness: differences in gene expression of native and introduced common ragweed (*Ambrosia artemisiifolia*) in stressful and benign environments. *Mol. Ecol.* 22, 2496–2510. doi: 10.1111/mec.12179

Hofmeister, B. T., Denkena, J., Colomé-Tatché, M., Shahryary, Y., Hazarika, R., Grimwood, J., et al. (2020). A genome assembly and the somatic genetic and epigenetic mutation rate in a wild long-lived perennial *Populus trichocarpa*. *Genome Biol.* 21:259.

Hofmeister, N. R., Werner, S. J., and Lovette, I. J. (2021). Environmental correlates of genetic variation in the invasive european starling in North America. *Mol. Ecol.* 30, 1251–1263. doi: 10.1111/mec.15806

Hu, C., Hart, S. N., Gnanaolivu, R., Huang, H., Lee, K. Y., Na, J., et al. (2021). A population-based study of genes previously implicated in breast cancer. *N. Engl. J. Med.* 384, 440–451.

Hu, Z., Ding, J., Ma, Z., Sun, R., Seoane, J. A., Scott Shaffer, J., et al. (2019). Quantitative evidence for early metastatic seeding in colorectal cancer. *Nat. Genet.* 51, 1113–1122. doi: 10.1038/s41588-019-0423-x

Ibrahim-Hashim, A., Luddy, K., Abrahams, D., Enriquez-Navas, P., Damgaci, S., Yao, J., et al. (2021). Artificial selection for host resistance to tumour growth and subsequent cancer cell adaptations: an evolutionary arms race. *Br. J. Cancer* 124, 455–465. doi: 10.1038/s41416-020-01110-1

Ibrahim-Hashim, A., Robertson-Tessi, M., Enriquez-Navas, P. M., Damaghi, M., Balagurunathan, Y., Wojtkowiak, J. W., et al. (2017). Defining cancer subpopulations by adaptive strategies rather than molecular properties provides novel insights into intratumoral evolution. *Cancer Res.* 77, 2242–2254. doi: 10.1158/0008-5472.can-16-2844

ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium (2020). Pan-cancer analysis of whole genomes. *Nature* 578, 82–93. doi: 10.1038/s41586-020-1969-6

Irimia, R. E., Hierro, J. L., Branco, S., Sotes, G., Cavieres, L. A., Eren, Ö, et al. (2021). Experimental admixture among geographically disjunct populations of an invasive plant yields a global mosaic of reproductive incompatibility and heterosis. *J. Ecol.* 109, 2152–2162. doi: 10.1111/1365-2745.13628

Jablonka, E. (2017). The evolutionary implications of epigenetic inheritance. *Interface Focus* 7:20160135. doi: 10.1098/rsfs.2016.0135

Jablonka, E., and Lamb, M. J. (1998). Epigenetic inheritance in evolution. *J. Evol. Biol.* 11, 159–183.

Jablonka, E., and Lamb, M. J. (1999). *Epigenetic Inheritance and Evolution: the Lamarckian Dimension*. Oxford: Oxford University Press.

Jablonka, E., and Raz, G. (2009). Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.* 84, 131–176. doi: 10.1086/598822

Jamal-Hanjani, M., Wilson, G. A., McGranahan, N., Birkbak, N. J., Watkins, T. B. K., Veeriah, S., et al. (2017). Tracking the evolution of non-small-cell lung cancer. *N. Engl. J. Med.* 376, 2109–2121.

Johannes, F., Porcher, E., Teixeira, F. K., Saliba-Colombani, V., Simon, M., Agier, N., et al. (2009). Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.* 5:e1000530. doi: 10.1371/journal.pgen.1000530

Johnson, K. E., Howard, G., Mo, W., Strasser, M. K., Lima, E. A. B. F., Huang, S., et al. (2019). Cancer cell population growth kinetics at low densities deviate from the exponential growth model and suggest an Allee effect. *PLoS Biol.* 17:e3000399. doi: 10.1371/journal.pbio.3000399

Joosse, S. A., Gorges, T. M., and Pantel, K. (2015). Biology, detection, and clinical implications of circulating tumor cells. *EMBO Mol. Med.* 7, 1–11. doi: 10.15252/emmm.201303698

Kadoch, C., and Crabtree, G. R. (2015). Mammalian SWI/SNF chromatin remodeling complexes and cancer: mechanistic insights gained from human genomics. *Sci. Adv.* 1:e1500447. doi: 10.1126/sciadv.1500447

Kandoth, C., McLellan, M. D., Vandin, F., Ye, K., Niu, B., Lu, C., et al. (2013). Mutational landscape and significance across 12 major cancer types. *Nature* 502, 333–339. doi: 10.1038/nature12634

Kang, Y., Siegel, P. M., Shu, W., Drobnjak, M., Kakonen, S. M., Cordón-Cardo, C., et al. (2003). A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 3, 537–5496. doi: 10.1016/s1535-6108(03)00132-6

Katzke, V. A., Kaaks, R., and Kühn, T. (2015). Lifestyle and Cancer Risk. *Cancer J.* 21, 104–110.

Keller, E. F. (2014). From gene action to reactive genomes. *J. Physiol.* 592, 2423–2429. doi: 10.1113/jphysiol.2014.270991

Keller, R. P., Lodge, D. M., and Finnoff, D. C. (2007). Risk assessment for invasive species produces net bioeconomic benefits. *Proc. Natl. Acad. Sci. USA* 104, 203–207. doi: 10.1073/pnas.0605787104

Kolbe, J. J., Glor, R. E., Rodríguez Schettino, L., Lara, A. C., Larson, A., and Losos, J. B. (2004). Genetic variation increases during biological invasion by a Cuban lizard. *Nature* 431, 177–181. doi: 10.1038/nature02807

Kooke, R., Morgado, L., Becker, F., van Eekelen, H., Hazarika, R., Zheng, Q., et al. (2019). Epigenetic mapping of the Arabidopsis metabolome reveals mediators of the epigenotype-phenotype map. *Genome Res.* 29, 96–106. doi: 10.1101/gr.232371.117

Kramer, A., and Sarnelle, O. (2008). Limits to genetic bottlenecks and founder events imposed by the allee effect. *Oecologia* 157, 561–569. doi: 10.1007/s00442-008-1105-z

Krueger-Hadfield, S. A., Kollars, N. M., Byers, J. E., Greig, T. W., Hammann, M., Murray, D. C., et al. (2016). Invasion of novel habitats uncouples haplo-diplontic life cycles. *Mol. Ecol.* 25, 3801–3816. doi: 10.1111/mec.13718

Kumar, S., and Weaver, V. M. (2009). Mechanics, malignancy, and metastasis: the force journey of a tumor cell. *Cancer Metastasis Rev.* 28, 113–127. doi: 10.1007/s10555-008-9173-4

Kuramochi, H., Nakajima, G., Kaneko, Y., Nakamura, A., Inoue, Y., Yamamoto, M., et al. (2012). Amphiregulin and Epiregulin mRNA expression in primary colorectal cancer and corresponding liver metastases. *BMC Cancer* 12:88.

Lai, Z., Gross, B. L., Zou, Y., Andrews, J., and Rieseberg, L. H. (2006). Microarray analysis reveals differential gene expression in hybrid sunflower species. *Mol. Ecol.* 15, 1213–1227. doi: 10.1111/j.1365-294x.2006.02775.x

Laitinen, R. A. E., and Nikoloski, Z. (2019). Genetic basis of plasticity in plants. *J. Exp. Bot.* 70, 739–745. doi: 10.1093/jxb/ery404

Lambert, A. W., Pattabiraman, D. R., and Weinberg, R. A. (2017). Emerging biological principles of metastasis. *Cell* 168, 670–691. doi: 10.1016/j.cell.2016.11.037

Ledford, H. (2010). Big science: the cancer genome challenge. *Nature* 464, 972–974. doi: 10.1038/464972a

Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends Ecol. Evol.* 17, 386–391. doi: 10.1016/s0169-5347(02)02554-5

Lee-Six, H., Øbro, N. F., Shepherd, M. S., Grossmann, S., Dawson, K., Belmonte, M., et al. (2018). Population dynamics of normal human blood inferred from somatic mutations. *Nature* 561, 473–478. doi: 10.1038/s41586-018-0497-0

Lee-Six, H., Olafsson, S., Ellis, P., Osborne, R. J., Sanders, M. A., Moore, L., et al. (2019). The landscape of somatic mutation in normal colorectal epithelial cells. *Nature* 574, 532–537. doi: 10.1038/s41586-019-1672-7

Levine, J. M., Adler, P. B., and Yelenik, S. G. (2004). A meta-analysis of biotic resistance to exotic plant invasions: biotic resistance to plant invasion. *Ecol. Lett.* 7, 975–989. doi: 10.1111/j.1461-0248.2004.00657.x

Levis, N. A., and Pfennig, D. W. (2016). Evaluating "plasticity-first" evolution in nature: key criteria and empirical approaches. *Trends Ecol. Evol.* 31, 563–574. doi: 10.1016/j.tree.2016.03.012

Liebhold, A. M., Berec, L., Brockerhoff, E. G., Epanchin-Niell, R. S., Hastings, A., Herms, D. A., et al. (2016). Eradication of invading insect populations: from concepts to applications. *Annu. Rev. Entomol.* 61, 335–352. doi: 10.1146/annurev-ento-010715-023809

Liebl, A. L., Schrey, A. W., Andrew, S. C., Sheldon, E. L., and Griffith, S. C. (2015). Invasion genetics: lessons from a ubiquitous bird, the house sparrow Passer domesticus. *Curr. Zool.* 61, 465–476. doi: 10.1093/czoolo/61.3.465

Lloyd, M. C., Cunningham, J. J., Bui, M. M., Gillies, R. J., Brown, J. S., and Gatenby, R. A. (2016). Darwinian dynamics of intratumoral heterogeneity: not solely random mutations but also variable environmental selection forces. *Cancer Res.* 76, 3136–3144. doi: 10.1158/0008-5472.can-15-2962

Lloyd, M. C., Gatenby, R. A., and Brown, J. S. (2017). "Ecology of the metastatic process," in *Ecology and Evolution of Cancer*, eds B. Ujvari, B. Roche, and F. Thomas (Amsterdam: Elsevier).

Lodge, D. M. (1993). Biological invasions: lessons for ecology. *Trends Ecol. Evol.* 8, 133–137. doi: 10.1016/0169-5347(93)90025-k

Lodge, D. M., Simonin, P. W., Burgiel, S. W., Keller, R. P., Bossenbroek, J. M., Jerde, C. L., et al. (2016). Risk analysis and bioeconomics of invasive species to inform policy and management. *Annu. Rev. Environ. Resour.* 41, 453–488.

Loeb, L. A. (2011). Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat. Rev. Cancer* 11, 450–457. doi: 10.1038/nrc3063

Loeb, L. A., Loeb, K. R., and Anderson, J. P. (2003). Multiple mutations and cancer. *Proc. Natl. Acad. Sci. USA* 100, 776–781.

Marin, P., Genitoni, J., Barloy, D., Maury, S., Gibert, P., Ghalambor, C. K., et al. (2020). Biological invasion: the influence of the hidden side of the (epi)genome. *Funct. Ecol.* 34, 385–400. doi: 10.1111/1365-2435.13317

Maron, J. L., and Marler, M. (2008). Effects of native species diversity and resource additions on invader impact. *Am. Nat.* 172(Suppl. 1), S18–S33.

Martincorena, I., and Campbell, P. J. (2015). Somatic mutation in cancer and normal cells. *Science* 349, 1483–1489. doi: 10.1126/science.aab4082

Martincorena, I., Fowler, J. C., Wabik, A., Lawson, A. R. J., Abascal, F., Hall, M. W. J., et al. (2018). Somatic mutant clones colonize the human esophagus with age. *Science* 362, 911–917. doi: 10.1126/science.aau3879

Martincorena, I., Roshan, A., Gerstung, M., Ellis, P., Van Loo, P., McLaren, S., et al. (2015). Tumor evolution. high burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 348, 880–886. doi: 10.1126/science.aaa6806

Matesanz, S., Rubio Teso, M. L., García-Fernández, A., and Escudero, A. (2017). Habitat fragmentation differentially affects genetic variation, phenotypic plasticity and survival in populations of a gypsum endemic. *Front. Plant Sci.* 8:843.

McGill, B. J., and Brown, J. S. (2007). Evolutionary game theory and adaptive dynamics of continuous traits. *Annu. Rev. Ecol. Evol. Syst.* 38, 403–435. doi: 10.1146/annurev.ecolsys.36.091704.175517

Moon, K., Blackman, D. A., and Brewer, T. D. (2015). Understanding and integrating knowledge to improve invasive species management. *Biol. Invasions* 17, 2675–2689. doi: 10.1007/s10530-015-0904-5

Moore, L., Leongamornlert, D., Coorens, T. H. H., Sanders, M. A., Ellis, P., Dentro, S. C., et al. (2020). The mutational landscape of normal human endometrial epithelium. *Nature* 580, 640–646.

Mounger, J., Ainouche, M. L., Bossdorf, O., Cavé-Radet, A., Li, B., Parepa, M., et al. (2021a). Epigenetics and the success of invasive plants. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 376:20200117. doi: 10.1098/rstb.2020.0117

Mounger, J., Teresa Boquete, M., Schmid, M. W., Granado, R., Robertson, M. H., Voors, S. A., et al. (2021b). Inheritance of DNA methylation differences in the mangrove Rhizophora mangle. *Evol. Dev.* 23, 351–374. doi: 10.1111/ede.12388

Müller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M. E., et al. (2001). Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410, 50–56.

Müller, G. B. (2017). Why an extended evolutionary synthesis is necessary. *Interface Focus* 7:20170015. doi: 10.1098/rsfs.2017.0015

Naciri, Y., and Linder, H. P. (2020). The genetics of evolutionary radiations. *Biol. Rev. Camb. Philos. Soc.* 95, 1055–1072. doi: 10.1111/brv.12598

Nam, A. S., Chaligne, R., and Landau, D. A. (2021). Integrating genetic and non-genetic determinants of cancer evolution by single-cell multi-omics. *Nat. Rev. Genet.* 22, 3–18. doi: 10.1038/s41576-020-0265-5

Narum, S. R., Buerkle, C. A., Davey, J. W., Miller, M. R., and Hohenlohe, P. A. (2013). Genotyping-by-sequencing in ecological and conservation genomics. *Mol. Ecol.* 22, 2841–2847. doi: 10.1111/mec.12350

Nature. (2020). The era of massive cancer sequencing projects has reached a turning point [Review of the era of massive cancer sequencing projects has reached a turning point]. *Nature* 578, 7–8. doi: 10.1038/d41586-020-00308-w

Nguyen, D. X., and Massagué, J. (2007). Genetic determinants of cancer metastasis. *Nat. Rev. Genet.* 8, 341–352. doi: 10.1038/nrg2101

Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., et al. (2010). Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* 15, 684–692. doi: 10.1016/j.tplants.2010.09.008

Nieto Feliner, G., Casacuberta, J., and Wendel, J. F. (2020). Genomics of evolutionary novelty in hybrids and polyploids. *Front. Genet.* 11:792.

Nik-Zainal, S., Van Loo, P., Wedge, D. C., Alexandrov, L. B., Greenman, C. D., Lau, K. W., et al. (2012). The life history of 21 breast cancers. *Cell* 149, 994–1007.

Nowell, P. C. (1976). The clonal evolution of tumor cell populations. *Science* 194, 23–28. doi: 10.1126/science.959840

Nunez-Mir, G. C., Liebhold, A. M., Guo, Q., Brockerhoff, E. G., Jo, I., Ordonez, K., et al. (2017). Biotic resistance to exotic invasions: its role in forest ecosystems, confounding artifacts, and future directions. *Biol. Invasions* 19, 3287–3299. doi: 10.1007/s10530-017-1413-5

Ortiz-Otero, N., Marshall, J. R., Lash, B. W., and King, M. R. (2020). Platelet mediated TRAIL delivery for efficiently targeting circulating tumor cells. *Nanoscale Adv.* 2, 3942–3953. doi: 10.1039/d0na00271b

Oshi, M., Katsuta, E., Yan, L., Ebos, J. M. L., Rashid, O. M., Matsuyama, R., et al. (2020). A Novel 4-Gene score to predict survival, distant metastasis and response to neoadjuvant therapy in breast cancer. *Cancers* 12:1148. doi: 10.3390/cancers12051148

Ossowski, S., Schneeberger, K., Lucas-Lledó, J. I., Warthmann, N., Clark, R. M., Shaw, R. G., et al. (2010). The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science* 327, 92–94. doi: 10.1126/science.1180677

Paape, T., Akiyama, R., Cereghetti, T., Onda, Y., Hirao, A. S., Kenta, T., et al. (2020). Experimental and field data support range expansion in an allopolyploid arabidopsis owing to parental legacy of heavy metal Hyperaccumulation. *Front. Genet.* 11:565854.

Paget, S. (1889). The distribution of secondary growths in cancer of the breast. *Lancet* 133, 571–573. doi: 10.1016/s0140-6736(00)49915-0

Palacio-López, K., and Gianoli, E. (2011). Invasive plants do not display greater phenotypic plasticity than their native or non-invasive counterparts: a meta-analysis. *Oikos* 120, 1393–1401. doi: 10.1111/j.1600-0706.2010.19114.x

Pantel, K., and Alix-Panabières, C. (2019). Liquid biopsy and minimal residual disease - latest advances and implications for cure. *Nat. Rev. Clin. Oncol.* 16, 409–424. doi: 10.1038/s41571-019-0187-3

Parkes, J. P., and Panetta, F. D. (2009). "Eradication of invasive species: progress and emerging issues in the 21st century," in *Invasive Species Management: a Handbook of Principles and Techniques. Techniques in Ecology and Conservation Series. Chapter 4*. Oxford: Oxford University Press. 47–60.

Patel, S. A., and Vanharanta, S. (2017). Epigenetic determinants of metastasis. *Mol. Oncol.* 11, 79–96. doi: 10.1016/j.molonc.2016.09.008

Patel, S. A., Rodrigues, P., Wesolowski, L., and Vanharanta, S. (2021). Genomic control of metastasis. *Br. J. Cancer* 124, 3–12.

Paterlini-Brechot, P., and Benali, N. L. (2007). Circulating tumor cells (CTC) detection: clinical impact and future directions. *Cancer Lett.* 253, 180–204. doi: 10.1016/j.canlet.2006.12.014

Paun, O., Verhoeven, K. J. F., and Richards, C. L. (2019). Opportunities and limitations of reduced representation bisulfite sequencing in plant ecological epigenomics. *New Phytol.* 221, 738–742. doi: 10.1111/nph.15388

Phillips, B. L., Brown, G. P., Travis, J. M. J., and Shine, R. (2008). Reid's paradox revisited: the evolution of dispersal kernels during range expansion. *Am. Nat.* 172, S34–S48.

Pienta, K. J., Hammarlund, E. U., Axelrod, R., Amend, S. R., and Brown, J. S. (2020a). Convergent evolution, evolving evolvability, and the origins of lethal cancer. *Mol. Cancer Res.* 18, 801–810. doi: 10.1158/1541-7786.mcr-19-1158

Pienta, K. J., Hammarlund, E. U., Axelrod, R., Brown, J. S., and Amend, S. R. (2020b). Poly-aneuploid cancer cells promote evolvability, generating lethal cancer. *Evol. Appl.* 13, 1626–1634. doi: 10.1111/eva.12929

Pienta, K. J., Hammarlund, E. U., Brown, J. S., Amend, S. R., and Axelrod, R. M. (2021). Cancer recurrence and lethality are enabled by enhanced survival and reversible cell cycle arrest of polyaneuploid cells. *Proc. Natl. Acad. Sci. USA* 118:e2020838118. doi: 10.1073/pnas.2020838118

Pigliucci, M. (2010). Genotype-phenotype mapping and the end of the "genes as blueprint" metaphor. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 365, 557–566. doi: 10.1098/rstb.2009.0241

Pimentel, D., Lach, L., Zuniga, R., and Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50, 53–65. doi: 10.1641/0006-3568(2000)050[0053:eaecon]2.3.co;2

Pintor, L. M., Brown, J. S., and Vincent, T. L. (2011). Evolutionary game theory as a framework for studying biological invasions. *Am. Nat.* 177, 410–423. doi: 10.1086/658149

Plutynski, A. (2021). Testing multi-task cancer evolution: How Do We test ecological hypotheses in cancer? *Front. Ecol. Evol.* 9:301.

Prentis, P. J., Wilson, J. R. U., Dormontt, E. E., Richardson, D. M., and Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends Plant Sci.* 13, 288–294. doi: 10.1016/j.tplants.2008.03.004

Pressley, M., Salvioli, M., Lewis, D. B., Richards, C. L., Brown, J. S., and Stankova, K. (2021). Evolutionary dynamics of drug resistance in cancer informs understanding of rapid evolution in natural systems. *Front. Ecol. Evol.* doi: 10.3389/fevo.2021.681121

Pyšek, P., and Richardson, D. M. (2010). Invasive species, environmental change and management, and health. *Annu. Rev. Environ. Resour.* 35, 25–55. doi: 10.1146/annurev-environ-033009-095548

Qu, X., Sandmann, T., Frierson, H., Fu, L., Fuentes, E., Walter, K., et al. (2016). Integrated genomic analysis of colorectal cancer progression reveals activation of EGFR through demethylation of the EREG promoter. *Oncogene* 35, 6403–6415. doi: 10.1038/onc.2016.170

Quilodrán, C. S., Tsoupas, A., and Currat, M. (2020). The spatial signature of introgression after a biological invasion with hybridization. *Front. Ecol. Evol.* 8:32120.

Rack, B., Schindlbeck, C., Jückstock, J., Andergassen, U., Hepp, P., Zwingers, T., et al. (2014). Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. *J. Natl. Cancer Inst.* 106:dju066.

Rahbari, R., Wuster, A., Lindsay, S. J., Hardwick, R. J., Alexandrov, L. B., Turki, S. A., et al. (2016). Timing, rates and spectra of human germline mutation. *Nat. Genet.* 48, 126–133. doi: 10.1038/ng.3469

Ramaswamy, S., Ross, K. N., Lander, E. S., and Golub, T. R. (2003). A molecular signature of metastasis in primary solid tumors. *Nat. Genet.* 33, 49–54. doi: 10.1038/ng1060

Regulations.gov (2020). Available Online at: https://www.federalregister.gov/documents/2020/12/15/2020-26734/removal-of-emerald-ash-borer-domestic-quarantine-regulations (accessed January 20, 2021).

Rejmánek, M. (1996). A theory of seed plant invasiveness: the first sketch. *Biol. Conserv.* 78, 171–181. doi: 10.1016/0006-3207(96)00026-2

Rejmánek, M. (2000). Invasive plants: approaches and predictions: invasive plants. *Austral Ecol.* 25, 497–506. doi: 10.1046/j.1442-9993.2000.01080.x

Richards, C. L., Alonso, C., Becker, C., Bossdorf, O., Bucher, E., Colomé-Tatché, M., et al. (2017). Ecological plant epigenetics: evidence from model and non-model species, and the way forward. *Ecol. Lett.* 20, 1576–1590. doi: 10.1111/ele.12858

Richards, C. L., and Pigliucci, M. (2020). Epigenetic inheritance. a decade into the extended evolutionary synthesis. *Paradigmi* 38, 463–494.

Richards, C. L., Bossdorf, O., and Pigliucci, M. (2010). What role does heritable epigenetic variation play in phenotypic evolution? *Bioscience* 60, 232–237. doi: 10.1525/bio.2010.60.3.9

Richards, C. L., Bossdorf, O., Muth, N. Z., Gurevitch, J., and Pigliucci, M. (2006). Jack of all trades, master of some? on the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* 9, 981–993. doi: 10.1111/j.1461-0248.2006.00950.x

Richards, C. L., Schrey, A. W., and Pigliucci, M. (2012). Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation. *Ecol. Lett.* 15, 1016–1025. doi: 10.1111/j.1461-0248.2012.01824.x

Richardson, D. M., and Pyšek, P. (2006). Plant invasions: merging the concepts of species invasiveness and community invasibility. *Prog. Phys. Geography Earth Environ.* 30, 409–431. doi: 10.1191/0309133306pp490pr

Richardson, D. M., Pysek, P., Rejmanek, M., Barbour, M. G., Panetta, F. D., and West, C. J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Divers. Distrib.* 6, 93–107. doi: 10.1046/j.1472-4642.2000.00083.x

Rieseberg, L. H., Raymond, O., Rosenthal, D. M., Lai, Z., Livingstone, K., Nakazato, T., et al. (2003). Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301, 1211–1216. doi: 10.1126/science.1086949

Risson, E., Nobre, A. R., Maguer-Satta, V., and Aguirre-Ghiso, J. A. (2020). The current paradigm and challenges ahead for the dormancy of disseminated tumor cells. *Nat. Cancer* 1, 672–680. doi: 10.1038/s43018-020-0088-5

Rius, M., Bourne, S., Hornsby, H. G., and Chapman, M. A. (2015a). Applications of next-generation sequencing to the study of biological invasions. *Curr. Zool.* 61, 488–504. doi: 10.1093/czoolo/61.3.488

Rius, M., Turon, X., Bernardi, G., Volckaert, F. A. M., and Viard, F. (2015b). Marine invasion genetics: from spatio-temporal patterns to evolutionary outcomes. *Biol. Invasions* 17, 869–885. doi: 10.1007/s10530-014-0792-0

Robertson, M., Alvarez, M., van Gurp, T., Wagemaker, C. A. M., Yu, F., Amador, D. M., et al. (2020). Combining epiGBS markers with long read transcriptome sequencing to assess differentiation associated with habitat in Reynoutria (aka Fallopia). *Biorxiv [preprint]* doi: 10.1101/2020.09.30.317966

Rodrigues, G., Hoshino, A., Kenific, C. M., Matei, I. R., Steiner, L., Freitas, D., et al. (2019). Tumour exosomal CEMIP protein promotes cancer cell colonization in brain metastasis. *Nat. Cell Biol.* 21, 1403–1412. doi: 10.1038/s41556-019-0404-4

Rollins, L. A., Richardson, M. F., and Shine, R. (2015). A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Mol. Ecol.* 24, 2264–2276. doi: 10.1111/mec.13184

Rollins, L. A., Woolnough, A. P., Fanson, B. G., Cummins, M. L., Crowley, T. M., Wilton, A. N., et al. (2016). Selection on mitochondrial variants occurs between and within Individuals in an expanding invasion. *Mol. Biol. Evol.* 33, 995–1007. doi: 10.1093/molbev/msv343

Rosenthal, D. M., Schwarzbach, A. E., Donovan, L. A., Raymond, O., and Rieseberg, L. H. (2002). Phenotypic differentiation between three ancient hybrid taxa and their parental species. *Int. J. Plant Sci.* 163, 387–398. doi: 10.1086/339237

Rotter, M. C., and Holeski, L. M. (2018). A meta-analysis of the evolution of increased competitive ability hypothesis: genetic-based trait variation and herbivory resistance trade-offs. *Biol. Invasions* 20, 2647–2660. doi: 10.1007/s10530-018-1724-1

Sansregret, L., and Swanton, C. (2017). The role of aneuploidy in cancer evolution. *Cold Spring Harbor Perspect. Med.* 7:a028373. doi: 10.1101/cshperspect.a028373

Sarma, R. R., Crossland, M. R., Eyck, H. J. F., DeVore, J. L., Edwards, R. J., Cocomazzo, M., et al. (2021). Intergenerational effects of manipulating DNA methylation in the early life of an iconic invader. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 376:20200125. doi: 10.1098/rstb.2020.0125

Sarma, R. R., Edwards, R. J., Crino, O. L., Eyck, H. J. F., Waters, P. D., Crossland, M. R., et al. (2020). Do epigenetic changes drive corticosterone responses to alarm cues in larvae of an invasive amphibian? *Integr. Compar. Biol.* 60, 1481–1494. doi: 10.1093/icb/icaa082

Sasaki, E., Kawakatsu, T., Ecker, J. R., and Nordborg, M. (2019). Common alleles of CMT2 and NRPE1 are major determinants of CHH methylation variation in *Arabidopsis thaliana*. *PLoS Genet.* 15:e1008492. doi: 10.1371/journal.pgen.1008492

Scascitelli, M., Whitney, K. D., Randell, R. A., King, M., Buerkle, C. A., and Rieseberg, L. H. (2010). Genome scan of hybridizing sunflowers from Texas (*Helianthus annuus* and H. debilis) reveals asymmetric patterns of introgression and small islands of genomic differentiation. *Mol. Ecol.* 19, 521–541. doi: 10.1111/j.1365-294x.2009.04504.x

Schild, T., Low, V., Blenis, J., and Gomes, A. P. (2018). Unique metabolic adaptations dictate distal organ-specific metastatic colonization. *Cancer Cell* 33, 347–354. doi: 10.1016/j.ccell.2018.02.001

Schlaepfer, M. A., Sax, D. F., and Olden, J. D. (2011). The potential conservation value of non-native species. *Conserv. Biol. J. Soc. Conserv. Biol.* 25, 428–437. doi: 10.1111/j.1523-1739.2010.01646.x

Schmid-Siegert, E., Sarkar, N., Iseli, C., Calderon, S., Gouhier-Darimont, C., Chrast, J., et al. (2017). Low number of fixed somatic mutations in a long-lived oak tree. *Nat. Plants* 3, 926–929. doi: 10.1038/s41477-017-0066-9

Schoen, D. J., and Schultz, S. T. (2019). Somatic mutation and evolution in plants. *Annu. Rev. Ecol. Evol. Syst.* 50, 49–73. doi: 10.1146/annurev-ecolsys-110218-024955

Schrey, A. W., Alvarez, M., Foust, C. M., Kilvitis, H. J., Lee, J. D., Liebl, A. L., et al. (2013). Ecological epigenetics: beyond MS-AFLP. *Integr. Compar. Biol.* 53, 340–350. doi: 10.1093/icb/ict012

Selechnik, D., Richardson, M. F., Shine, R., DeVore, J. L., Ducatez, S., and Rollins, L. A. (2019). Increased adaptive variation despite reduced overall genetic diversity in a rapidly adapting invader. *Front. Genet.* 10:1221.

Shan, S., Boatwright, J. L., Liu, X., Chanderbali, A. S., Fu, C., Soltis, P. S., et al. (2020). Transcriptome dynamics of the inflorescence in reciprocally formed allopolyploid *Tragopogon miscellus* (Asteraceae). *Front. Genet.* 11:888.

Sharov, A. A., Leonard, D., Liebhold, A. M., Roberts, E. A., and Dickerson, W. (2002). "Slow The Spread": a national program to contain the Gypsy moth. *J. Forestry* 100, 30–36.

Shi, W., Chen, X., Gao, L., Xu, C.-Y., Ou, X., Bossdorf, O., et al. (2018). Transient stability of epigenetic population differentiation in a clonal invader. *Front. Plant Sci.* 9:1851.

Shine, R. (2018). *Cane Toad Wars*. Berkeley, CA: University of California Press.

Shine, R., Brown, G. P., and Phillips, B. L. (2011). An evolutionary process that assembles phenotypes through space rather than through time. *Proc. Natl. Acad. Sci. USA* 108, 5708–5711. doi: 10.1073/pnas.1018989108

Short, J., Bradshaw, S. D., Giles, J., Prince, R. I. T., and Wilson, G. R. (1992). Reintroduction of macropods (Marsupialia: Macropodoidea) in Australia—a review. *Biol. Conserv.* 62, 189–204. doi: 10.1016/0006-3207(92)91047-v

Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annu. Rev. Ecol. Evol. Syst.* 40, 81–102. doi: 10.1146/annurev.ecolsys.110308.120304

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., et al. (2013). Impacts of biological invasions: what's what and the way forward. *Trends Ecol. Evol.* 28, 58–66. doi: 10.1016/j.tree.2012.07.013

Snyder, M. R., and Stepien, C. A. (2017). Genetic patterns across an invasion's history: a test of change versus stasis for the Eurasian round goby in North America. *Mol. Ecol.* 26, 1075–1090. doi: 10.1111/mec.13997

Somarelli, J. A. (2021). The hallmarks of cancer as ecologically driven phenotypes. *Front. Ecol. Evol.* 9:226.

Sommer, R. J. (2020). Phenotypic plasticity: from theory and genetics to current and future challenges. *Genetics* 215, 1–13. doi: 10.1534/genetics.120.303163

Sotka, E. E., Baumgardner, A. W., Bippus, P. M., Destombe, C., Duermit, E. A., Endo, H., et al. (2018). Combining niche shift and population genetic analyses predicts rapid phenotypic evolution during invasion. *Evol. Appl.* 11, 781–793. doi: 10.1111/eva.12592

Sottoriva, A., Barnes, C. P., and Graham, T. A. (2017). Catch my drift? Making sense of genomic intra-tumour heterogeneity. *Biochim. Biophys. Acta Rev. Cancer* 1867, 95–100. doi: 10.1016/j.bbcan.2016.12.003

Srivastava, S., Ghosh, S., Kagan, J., and Mazurchuk, R. (2018). The PreCancer Atlas (PCA). *Trends Cancer* 4, 513–514. doi: 10.1016/j.trecan.2018.06.003

Stajic, D., and Jansen, L. (2021). Empirical evidence for epigenetic inheritance driving evolutionary adaptation. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* doi: 10.1098/rstb.2020.0121

Stapley, J., Santure, A. W., and Dennis, S. R. (2015). Transposable elements as agents of rapid adaptation may explain the genetic paradox of invasive species. *Mol. Ecol.* 24, 2241–2252. doi: 10.1111/mec.13089

Stepien, C. A., Brown, J. E., Neilson, M. E., and Tumeo, M. A. (2005). Genetic diversity of invasive species in the Great lakes versus their Eurasian source populations: insights for risk analysis. *Risk Anal.* 25, 1043–1060. doi: 10.1111/j.1539-6924.2005.00655.x

Stewart, N. C., Tranel, P. J., Horvath, D. P., Anderson, J. V., Rieseberg, L. H., Westwood, J. H., et al. (2009). Evolution of weediness and invasiveness: charting the course for weed genomics. *Weed Sci.* 57, 451–462. doi: 10.1614/ws-09-011.1

Stratton, M. R. (2011). Exploring the genomes of cancer cells: progress and promise. *Science* 331, 1553–1558. doi: 10.1126/science.1204040

Strong, D. R., and Ayres, D. R. (2013). Ecological and evolutionary misadventures of spartina. *Annu. Rev. Ecol. Evol. Syst.* 44, 389–410. doi: 10.1146/annurev-ecolsys-110512-135803

Stuart, K. C., Cardilini, A. P. A., Cassey, P., Richardson, M. F., Sherwin, W. B., Rollins, L. A., et al. (2020). Signatures of selection in a recent invasion reveal adaptive divergence in a highly vagile invasive species. *Mol. Ecol.* 30, 1419–1434. doi: 10.1111/mec.15601

Stupack, D. G., Teitz, T., Potter, M. D., Mikolon, D., Houghton, P. J., Kidd, V. J., et al. (2006). Potentiation of neuroblastoma metastasis by loss of caspase-8. *Nature* 439, 95–99. doi: 10.1038/nature04323

Sultan, S. E. (2015). *Organism and Environment: Ecological Development, Niche Construction, and Adaptation*. Oxford: Oxford University Press.

Sun, Y., Beuchat, C., and Müller-Schärer, H. (2020). Is biocontrol efficacy rather driven by the plant or the antagonist genotypes? a conceptual bioassay approach. *Working Paper Series* 63, 81–10062. doi: 10.3897/neobiota.63.54962

Szczurek, E., Krüger, T., Klink, B., and Beerenwinkel, N. (2020). A mathematical model of the metastatic bottleneck predicts patient outcome and response to cancer treatment. *PLoS Comput. Biol.* 16:e1008056. doi: 10.1371/journal.pcbi.1008056

Tepolt, C. K., and Palumbi, S. R. (2020). Rapid adaptation to temperature via a potential genomic island of divergence in the invasive green crab, *Carcinus maenas*. *Front. Ecol. Evol.* 8:411.

Teschendorff, A. E., and Feinberg, A. P. (2021). Statistical mechanics meets single-cell biology. *Nat. Rev. Genet.* 22, 459–476. doi: 10.1038/s41576-021-00341-z

Theoharides, K. A., and Dukes, J. S. (2007). Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytol.* 176, 256–273. doi: 10.1111/j.1469-8137.2007.02207.x

Thuiller, W., Gallien, L., Boulangeat, I., De Bello, F., Münkemüller, T., Roquet, C., et al. (2010). Resolving Darwin's naturalization conundrum: a quest for evidence: resolving Darwin's naturalization conundrum. *Divers. Distrib.* 16, 461x–475x.

Timp, W., and Feinberg, A. P. (2013). Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. *Nat. Rev. Cancer* 13, 497–510. doi: 10.1038/nrc3486

Tissot, T., Massol, F., Ujvari, B., Alix-Panabieres, C., Loeuille, N., and Thomas, F. (2019). Metastasis and the evolution of dispersal. *Proc. R. Soc. B Biol. Sci.* 286:20192186186.

Tsherniak, A., Vazquez, F., Montgomery, P. G., Weir, B. A., Kryukov, G., Cowley, G. S., et al. (2017). Defining a cancer dependency map. *Cell* 170, 564–576.e16.

Turajlic, S., Xu, H., Litchfield, K., Rowan, A., Chambers, T., Lopez, J. I., et al. (2018). Tracking cancer evolution reveals constrained routes to metastases: TRACERx renal. *Cell* 173, 581–594.e12.

Van Buskirk, J., and Steiner, U. K. (2009). The fitness costs of developmental canalization and plasticity. *J. Evol. Biol.* 22, 852–860. doi: 10.1111/j.1420-9101.2009.01685.x

Van de Peer, Y., Mizrachi, E., and Marchal, K. (2017). The evolutionary significance of polyploidy. *Nat. Rev. Genet.* 18, 411–424. doi: 10.1038/nrg.2017.26

van Kleunen, M., Bossdorf, O., and Dawson, W. (2018). The ecology and evolution of alien plants. *Annu. Rev. Ecol. Evol. Syst.* 49, 25–47. doi: 10.1146/annurev-ecolsys-110617-062654

van Kleunen, M., Dawson, W., and Maurel, N. (2015). Characteristics of successful alien plants. *Mol. Ecol.* 24, 1954–1968. doi: 10.1111/mec.13013

van Riemsdijk, I., Arntzen, J. W., Bucciarelli, G., McCartney-Melstad, E., Rafajlović, M., Scott, P. A., et al. (2020). Spatial variation in introgression along a toad hybrid zone in France. *Biorxiv [preprint]* doi: 10.1101/746073

van Riemsdijk, I., van Nieuwenhuize, L., Martínez-Solano, I., Arntzen, J. W., and Wielstra, B. (2018). Molecular data reveal the hybrid nature of an introduced population of banded newts (Ommatotriton) in Spain. *Conserv. Genet.* 19, 249–254. doi: 10.1007/s10592-017-1004-0

van Steenderen, C. J. M., Paterson, I. D., Edwards, S., and Day, M. D. (2021). Addressing the red flags in cochineal identification: the use of molecular techniques to identify cochineal insects that are used as biological control agents for invasive alien cacti. *Biol. Control Theory Appl. Pest Manag.* 152:104426. doi: 10.1016/j.biocontrol.2020.104426

Vandepitte, K., de Meyer, T., Helsen, K., van Acker, K., Roldán-Ruiz, I., Mergeay, J., et al. (2014). Rapid genetic adaptation precedes the spread of an exotic plant species. *Mol. Ecol.* 23, 2157–2164. doi: 10.1111/mec.12683

VanWallendael, A., Alvarez, M., and Franks, S. J. (2020). Patterns of population genomic diversity in the invasive Japanese knotweed species complex. *Am. J. Bot.* 108, 857–886. doi: 10.1002/ajb2.1653

Verhoeven, K. J. F., and Preite, V. (2014). Epigenetic variation in asexually reproducing organisms. *Evolution* 68, 644–655. doi: 10.1111/evo.12320

Verhoeven, K. J. F., Jansen, J. J., Van Dijk, P. J., and Biere, A. (2010). Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol.* 185, 1108–1118. doi: 10.1111/j.1469-8137.2009.03121.x

Vittecoq, M., Giraudeau, M., Sepp, T., Marcogliese, D. J., Klaassen, M., Renaud, F., et al. (2018). Turning natural adaptations to oncogenic factors into an ally in the war against cancer. *Evol. Appl.* 11, 836–844. doi: 10.1111/eva.12608

Vogelstein, B., Papadopoulos, N., Velculescu, V. E., Zhou, S., Diaz, L. A., and Kinzler, K. W. (2013). Cancer genome landscapes. *Science* 339, 1546–1558.

Vonholdt, B. M., Pollinger, J. P., Lohmueller, K. E., Han, E., Parker, H. G., Quignon, P., et al. (2010). Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. *Nature* 464, 898–902. doi: 10.1038/nature08837

Wegner, K. M., Lokmer, A., and John, U. (2020). Genomic and transcriptomic differentiation of independent invasions of the pacific oyster *Crassostrea gigas*. *Front. Ecol. Evol.* 8:371.

Weisenberger, D. J., Siegmund, K. D., Campan, M., Young, J., Long, T. I., Faasse, M. A., et al. (2006). CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat. Genet.* 38, 787–793. doi: 10.1038/ng1834

Wells, A., Grahovac, J., Wheeler, S., Ma, B., and Lauffenburger, D. (2013). Targeting tumor cell motility as a strategy against invasion and metastasis. *Trends Pharmacol. Sci.* 34, 283–289. doi: 10.1016/j.tips.2013.03.001

Wendel, J. F., Jackson, S. A., Meyers, B. C., and Wing, R. A. (2016). Evolution of plant genome architecture. *Genome Biol.* 17:37.

West-Eberhard, M. J. (2005). Developmental plasticity and the origin of species differences. *Proc. Natl. Acad. Sci. USA* 102(Suppl. 1), 6543–6549. doi: 10.1073/pnas.0501844102

Whitney, K. D., Broman, K. W., Kane, N. C., Hovick, S. M., Randell, R. A., and Rieseberg, L. H. (2015). Quantitative trait locus mapping identifies candidate alleles involved in adaptive introgression and range expansion in a wild sunflower. *Mol. Ecol.* 24, 2194–22114. doi: 10.1111/mec.13044

Williams, M. J., Sottoriva, A., and Graham, T. A. (2019). Measuring clonal evolution in cancer with genomics. *Annu. Rev. Genomics Hum. Genet.* 20, 309–329. doi: 10.1146/annurev-genom-083117-021712

Xie, Z., Wang, L., Wang, L., Wang, Z., Lu, Z., Tian, D., et al. (2016). Mutation rate analysis via parent-progeny sequencing of the perennial peach. I. a low rate in woody perennials and a higher mutagenicity in hybrids. *Proc. Biol. Sci.* 283:20161016. doi: 10.1098/rspb.2016.1016

Xu, G., Lyu, J., Li, Q., Liu, H., Wang, D., Zhang, M., et al. (2020). Evolutionary and functional genomics of DNA methylation in maize domestication and improvement. *Nat. Commun.* 11:5539. doi: 10.1038/s41467-020-19333-4

Yap, T. A., Omlin, A., and de Bono, J. S. (2013). Development of therapeutic combinations targeting major cancer signaling pathways. *J. Clin. Oncol.* 31, 1592–1605. doi: 10.1200/jco.2011.37.6418

Yates, L. R., Knappskog, S., Wedge, D., Farmery, J. H. R., Gonzalez, S., Martincorena, I., et al. (2017). Genomic evolution of breast cancer metastasis and relapse. *Cancer Cell* 32, 169–184.e705.

Yoder, A. D., and Tiley, G. P. (2021). The challenge and promise of estimating the de novo mutation rate from whole-genome comparisons among closely related individuals. *Mol. Ecol.* doi: 10.1111/mec.16007

Yoo, M.-J., Liu, X., Pires, J. C., Soltis, P. S., and Soltis, D. E. (2014). Nonadditive gene expression in polyploids. *Annu. Rev. Genet.* 48, 485–517. doi: 10.1146/annurev-genet-120213-092159

Yu, M., Stott, S., Toner, M., Maheswaran, S., and Haber, D. A. (2011). Circulating tumor cells: approaches to isolation and characterization. *J. Cell Biol.* 192, 373–382. doi: 10.1083/jcb.201010021

Zahir, N., Sun, R., Gallahan, D., Gatenby, R. A., and Curtis, C. (2020). Characterizing the ecological and evolutionary dynamics of cancer. *Nat. Genet.* 52, 759–767. doi: 10.1038/s41588-020-0668-4

Zhang, Y. M., Vitone, T. R., Storer, C. G., Payton, A. C., Dunn, R. R., Hulcr, J., et al. (2019). From pavement to population genomics: characterizing a long-established non-native ant in north america through citizen science and ddRADseq. *Front. Ecol. Evol.* 7:453.

Zhang, Y.-Y., Parepa, M., Fischer, M., and Bossdorf, O. (2016). "Epigenetics of colonizing species? a study of japanese knotweed in central Europe," in *Invasion Genetics: The Baker and Stebbins Legacy*, eds S. Barrett, R. I. Colautti, K. M. Dlugosch, and L. H. Rieseberg (Hoboken, NJ: Wiley-Blackwell), 328–340. doi: 10.1002/9781119072799.ch19

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The handling editor declared a past collaboration with one of the authors, JB.

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frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 29 September 2021
doi: 10.3389/fevo.2021.697618

# Coexistence of "Cream Skimmer" and "Crumb Picker" Phenotypes in Nature and in Cancer

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OPEN ACCESS

**Edited by:**
*Jason Somarelli, Duke University Medical Center, United States*

**Reviewed by:**
*Andrew Letten, The University of Queensland, Australia*
*Francisco Garcia-Gonzalez, Estación Biológica de Doñana (EBD), Spain*

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**Specialty section:**
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

**Received:** *20 April 2021*
**Accepted:** *03 September 2021*
**Published:** *29 September 2021*

**Citation:**
*Huntly N, Freischel AR, Miller AK, Lloyd MC, Basanta D and Brown JS (2021) Coexistence of "Cream Skimmer" and "Crumb Picker" Phenotypes in Nature and in Cancer. Front. Ecol. Evol. 9:697618. doi: 10.3389/fevo.2021.697618*

Over 40 years ago, seminal papers by Armstrong and McGehee and by Levins showed that temporal fluctuations in resource availability could permit coexistence of two species on a single resource. Such coexistence results from non-linearities or non-additivities in the way resource supply translates into fitness. These reflect trade-offs where one species benefits more than the other during good periods and suffers more (or does less well) than the other during less good periods, be the periods stochastic, unstable population dynamics, or seasonal. Since, coexistence based on fluctuating conditions has been explored under the guises of "grazers" and "diggers," variance partitioning, relative non-linearity, "opportunists" and "gleaners," and as the storage effect. Here we focus on two phenotypes, "cream skimmers" and "crumb pickers," the former having the advantage in richer times and the latter in less rich times. In nature, richer and poorer times, with regular or stochastic appearances, are the norm and occur on many time scales. Fluctuations among richer and poorer times also appear to be the norm in cancer ecosystems. Within tumors, nutrient availability, oxygen, and pH can fluctuate stochastically or periodically, with swings occurring over seconds to minutes to hours. Despite interest in tumor heterogeneity and how it promotes the coexistence of different cancer cell types, the effects of fluctuating resource availability have not been explored for cancer. Here, in the context of pulsed resources, we (1) develop models of foraging consumers who experience pulsed resources to examine four types of trade-offs that can promote coexistence of phenotypes that do relatively better in richer versus in poorer times, (2) establish that conditions in tumors are conducive for this mechanism, (3) propose and empirically explore biomarkers indicative of the two phenotypes (HIF-1, GLUT-1, CA IX, CA XII), and (4) and compare cream skimmer and crumb picker biology and ecology in nature and cancer to provide cross-disciplinary insights into this interesting, and, we argue, likely very common, mechanism of coexistence.

**Keywords: coexistence, biodiversity, foraging behavior, fixed and variable costs, fluctuating environment, cream skimmer, crumb picker, non-equilibrium coexistence**

## INTRODUCTION

Biodiversity, the presence of many phenotypes and species, is a ubiquitous feature of nature. Species coexist by preferentially consuming different foods (diet separation), occupying different times and places (habitat separation), or varying in their capacities to avoid hazards and exploit opportunities (predation-based or food-safety trade-offs) (e.g., Pulliam, 1974; Schoener, 1974; Werner and Hall, 1977; Kotler and Brown, 1988; Huntly, 1991; Morris, 2003). Community ecologists construct theories and models to understand how biodiversity might exist, and then test empirically what mechanisms do promote coexistence of different species. Diversity also seems the norm in cancers, where cancer biologists recognize much variety among the cancer cells that inhabit tumor ecosystems within patients. Metrics generally involve genetic and molecular variation, but much of this can be clustered into what appear to be distinct cancer cell phenotypes (e.g., Amaro et al., 2016; Yeo and Guan, 2017; Wooten et al., 2019; Iravani et al., 2021). These types may coexist within tumor microenvironments, across whole tumors, or among tumors within a single patient (Lloyd et al., 2016). We suggest here that the different cancer cell types may equate to biodiversity in nature. Kotler and Brown (2020) have proposed cancer community ecology as a parallel to community ecology in nature to study the mechanisms that promote a diversity of cancer cell types.

The competitive exclusion principle (Hardin, 1961) has provided a basis for understanding how species coexist. It states that no two species can coexist by occupying the same niche at the same place and time. To add rigor, ecologists have noted that for two species to coexist there must be at least two (or a continuum of) limiting factors. These limiting factors can take the form of resources or hazards. For instance, two consumer species can coexist if one is more successful at exploiting resource A, the other is the more successful with resource B, and both A and B are sufficiently abundant to support the species. The two species coexist by trade-offs in their abilities to harvest and use the two resources. Alternatively, the two species can coexist if the species that is better at exploiting resource A is also more vulnerable to predation risk, promoting coexistence by a food-safety trade-off.

An early challenge to the competitive exclusion principle was the Paradox of the Plankton (Hutchinson, 1961). In many aquatic ecosystems, the number of limiting resources (e.g., nitrogen, phosphorus, organic carbon, carbon dioxide) seemed much less than the number of coexisting phytoplankton species. Subtle habitat selection, trade-offs in absorbing different sizes and states of micro- and macro-molecules, and threats from numerous species of predators have been proposed to reconcile the paradox (e.g., Litchman and Klausmeier, 2008; Salcher, 2014). However, Hutchinson suggested that fluctuation in conditions over time might itself contribute to coexistence of biodiverse plankton. Fluctuations are reflected in the higher statistical moments of resource availabilities, the variance and covariance, which can be viewed as reflecting potentially separate "resources" (Levins, 1979; Chesson and Huntly, 1989; Chesson, 1994). Such resources could include, for instance, seeds, for a granivore, at high versus low abundance (Brown, 1989b; Kotler et al., 1993), abiotic essential nutrients for plankton at different seasonal temperatures (Descamps-Julien and Gonzalez, 2005), the prey of cold versus warm water fishes in summer versus winter (McMeans et al., 2020), or water in a year of early abundant rainfall or of drought (Chesson et al., 2004, 2013; Hallett et al., 2019).

Armstrong and McGehee (1976, 1980) provided early mathematical models of how temporal resource fluctuations could permit coexistence of more species than resources, even when both rank good and bad times the same (see Koch, 1974). Trade-offs between the relative foraging, recruitment, or survival success of two species over temporal fluctuations in environmental conditions make coexistence of two species on a single resource possible. One species does better than the other during more favorable periods, while doing worse than the other during less favorable periods. Various models have explored behaviors that can underly species coexistence when resource availability fluctuates over time. These models have taken a number of forms. including endogenously generated non-equilibrium consumer-resource dynamics, exogenously generated seasonal or pulsed resource renewal, and the storage effect (e.g., Armstrong and McGehee, 1976, 1980; Chesson and Warner, 1981; Chesson and Huntly, 1988, 1997; Brown, 1989a; Abrams and Holt, 2002; Abrams et al., 2003; Abrams, 2004; Xiao and Fussmann, 2013). All of these models include times of relatively richer and relatively poorer conditions. The contrasting phenotypes have been referred to as "opportunist" and "gleaner" (Grover, 1990), "grazers" and "diggers" (Richards et al., 2000), or "cream skimmers" and "crumb pickers" (Brown et al., 1994; Jones et al., 2001; Bonsall et al., 2002). We shall use the last of these three as has previously been used in the cancer literature (Gillies et al., 2018; Kotler and Brown, 2020). Here, we are specifically interested in fluctuations in resource availability as a mechanism of coexistence in nature and in the potential of this mechanism to explain some of the variation in coexisting phenotypes of cancer cells.

In general, coexistence on seasonal or otherwise pulsed resources can happen in at least two ways. First, the coexistence of two consumer species is possible if one has the higher foraging efficiency at high resource abundances, while the other has the higher efficiency at low resource abundances (Stewart and Levin, 1973; Abrams, 1984). Foraging efficiency in this case is the ratio of foraging benefits to total foraging costs. Second, foraging costs may be divisible into fixed and variable costs whereby a forager can avoid the variable costs of foraging through resting, ceasing activity, or dormancy. Coexistence on pulsed resources becomes possible if there is a trade-off between fixed and variable costs (Brown, 1989a). Under the circumstances, these tradeoffs are necessary but not sufficient. Coexistence also requires that each species depletes resources in a manner that is more favorable for the other species than itself.

We center in this paper on foraging trade-offs that could promote coexistence of cream-skimmer versus crumb-picker consumer phenotypes when the environment has pulsed resource supply followed by depletion through consumption. We explore the potential for behavioral trade-offs along a continuum of environmental favorability that make a cream

skimmer and crumb picker relatively better than the other during richer and poorer times, respectively. In nature, many examples of such rich/poor environmental conditions and cream-skimmer/crumb-picker phenotypes or species have been identified, including hummingbirds and bees, nectar yeasts, woodland rodents, large and small desert rodents, annual and perennial plants, grasses and forbs, slow- and fast-growing mosses, and various planktonic and intertidal organisms (Schaffer et al., 1979; Brown et al., 1981; Kotler and Brown, 1990; Wolfe, 1996; Wilson et al., 1999; Descamps-Julien and Gonzalez, 2005; Cermeno et al., 2011; McNickle et al., 2016; Letten et al., 2018; Oke and Turetsky, 2020). The basic idea of cream skimmer and crumb picker trade-offs can generalize to continua of richer and poorer conditions and to larger numbers of coexisting species, for which examples include diverse desert annuals (Angert et al., 2009; Chesson et al., 2013), grassland plants (Zepeda and Martorell, 2019), plankton (Huisman and Weissing, 1999; Huisman et al., 2001), and acorn-inhabiting weevils (Venner et al., 2011).

In cancer ecosystems, variation in environmental favorability and resource abundances also appears to be the norm. Within tumors, nutrient availability, oxygen, and pH can fluctuate stochastically or periodically, with swings occurring on scales of seconds to minutes to hours and varying among spatial locations (Michiels et al., 2016; Gillies et al., 2018; Saxena and Jolly, 2019). Also in cancer ecosystems, diversity or heterogeneity of cell types within tumors is the norm and correlates with resistance of tumors to therapies (Marusyk and Polyak, 2010; Robertson-Tessi et al., 2015; Lloyd et al., 2016). Despite considerable interest in tumor heterogeneity and how it may promote coexistence of different cell types, coexistence of cancer cell types as cream skimmers and crumb pickers has not been explored. Here, we (1) use consumer-resource models to examine several types of trade-offs that can promote coexistence, (2) establish the conditions in tumors conducive for such a mechanism, (3) propose and explore biomarkers indicative of cream-skimmer and crumb-picker phenotypes (HIF-1; GLUT-1; CA IX; CA XII, and others), and (4) and compare actual and potential examples of these phenotypes in nature around us and in cancer.

**TABLE 1 |** Model parameters, variables, and critical values.

| Parameter | Definition | Units |
|---|---|---|
| $b$ | Conversion factor of net profit rate into per capita growth | Per resource |
| $a$ | Per capita encounter rate | Per time |
| $h$ | Handling/processing time of one unit of resource | Time per resource |
| $c$ | Per capita cost of existence | Resources per time |
| $r$ | Resource renewal rate | Per time |
| $K$ | Maximum amount of resource that may be present in a system (resource carrying capacity) | Resources |
| $R_0$ | The amount of resource pulsed in the system | Resources |
| $T$ | Pulse time | Time |
| **Variable** | | |
| $R$ | Resource density | Resource density or concentration |
| $X$ | Population density | Consumer density |
| **Values** | | |
| $R_{12}$ | Concentration of resource above which the cream-skimmers exclude the crumb-pickers | Resources |
| $R_{21}$ | Concentration of resource below which the crumb-pickers exclude the cream-skimmers | Resources |
| $R_i^*$ | Abundance of resources required to maintain a consumer population at steady state ($dx/dt = 0$) | Resources |
| $x_i^*$ | The steady state of a population when $R = R_i^*$ | Consumers |
| $R'$ | Resource level at which the net profit rate of the cream-skimmer is equal to that of the crumb-picker | Resources |
| $H(R)$ | Harvest rate | Resources per time |
| $\pi$ | Net profit rate | Resources per time |

# METHODS AND RESULTS: ANALYSIS OF A CONSUMER-RESOURCE MODEL

## Continuous Resource Renewal

We imagine two consumer species (cancer cell phenotypes) harvesting (nutrient uptake in cancer) a single resource. We start with continuous resource renewal and the following consumer-resource dynamics:

$$\frac{1}{x_i}\frac{dx_i}{dt} = b_i\left[\frac{a_i R}{1 + a_i h_i R} - c_i\right]$$

$$\frac{dR}{dt} = r\,(K - R) - \sum \frac{x_i a_i R}{1 + a_i h_i R}$$

where $x_i$ are the population densities of consumer species $i = 1,2$, and $R$ is the density of resources (see **Table 1** for list of model variables and parameters).

Net profit rate, the difference between resource harvest rate and the cost of existence, $c_i$ (in units of resources per time), determines whether the per capita population growth rate of a consumer species is positive or negative. The conversion factor of net profit rate into per capita growth, $b_i$, scales the rate of growth or decline. The consumers harvest resources by encountering and then handling them, where $a_i$ describes the probability of resource encounter per unit time (encounter rate, or attack rate), and $h_i$ describes the time taken to handle an encountered resource item. In cancer cells, encounter rates (per time) and handling times (time per item or molecule) with extracellular molecules vary and are mediated by the presence, number, and speed of carrier and channel proteins (in the case of facilitated diffusion) or transporter molecules (in the case of active transport) (e.g., Perfahl et al., 2013; Lisan and Langhans, 2015). Other forms of encounter and uptake that require even more handling effort include receptor-mediated endocytosis and pinocytosis involving the formation of vesicles and engulfment, respectively.

We assume that the consumers' harvest rates, $H$ ($R$), follow Holling's (1959) disc equation (type II functional response), taking the form of a Michaelis Menten or Monod equation:

$$H_i(R) = \frac{a_i R}{1 + a_i h_i R}$$

With continuous resource renewal, we have something akin to a chemostat where the resource flows into the system at some rate, $r$, and at some concentration, $K$. Resources (but not the consumers) are lost via outflow and consumption by the consumers. In the absence of consumers, the resource density (or concentration) would equilibrate at $R = K$. With consumption, the resource equilibrates at a lower density. For solid tumor cancers, the tumor can be viewed as a somewhat viscous chemostat, with blood flow providing and removing resources (among other bloodborne normal cells and metabolites).

Each consumer species will have a subsistence level of resource, $R_i^*$, above which it experiences a positive growth rate and below which its population declines. This subsistence level is species-specific. It is the value of $R$ such that a species' net profit rate from foraging is 0:

$$R_i^* = \frac{c_i}{a_i(1 - c_i h_i)}$$

This subsistence level increases with handling time, $h_i$, and the cost of existence, $c_i$, and decreases with encounter rate, $a_i$. Note that for the resource to have any value to the consumer $c_i h_i < 1$.

With just a single consumer species, its population size will achieve an equilibrium, $x_i^*$ (when $x_j = 0$), such that the equilibrium resource availability has been driven to the consumer species' $R_i^*$:

$$x_i^* = r\left(\frac{K}{c_i} - \frac{1}{a_i(1 - c_i h_i)}\right)$$

As expected, the equilibrium number of a single consumer species will increase linearly with the flow rate of resources into the system, $r$, and the concentration of those resources, $K$. For $x_i^* > 0$, the incoming resource concentration must be higher than the consumer species' $R_i^*$: $K > R_i^*$.

### Key Results

If the incoming resource concentration is too low, $K < R_i^*$ for $i = 1,2$, then neither consumer species can exist (i.e., the necrotic zone in a tumor). If the $R_2^* < K < R_1^*$, then by default only consumer species 2 can exist in the community. If $K > R_i^*$ for $i = 1,2$, then the consumer with the lower $R^*$ will outcompete the other. Hence, at most, just one consumer species can exist in this community.

### Significance

All of the above are well-known results from consumer-resource theory. But, they provide the jumping off point for considering the effect of a pulsed (batch chemostat) rather than continuously supplied (continuous flow chemostat) resource. All of these results emanate from three foraging parameters: encounter rate, handling time, and cost of existence. This consumer-resource approach is relatively unexplored in cancer (Amend et al., 2018), and may be quite applicable to 3-D spheroid (Carvalho et al., 2015; Ravi et al., 2015; Agrawal et al., 2021) and organoid culture experiments (Lo et al., 2020; Schuster et al., 2020), and to mouse experiments involving competition between different cancer cell lines (Di Gregorio et al., 2016; Parker et al., 2020).

## Pulsed Resource: Encounter Rate and Handling Time Trade-Off

Pulsed nutrient renewal is a feature of nature (daily or seasonal pulses), tumors (intra-tumoral cycles of blood flow, resource availability, and hypoxia), and cell culture experiments (regular changes to the growth medium every so many days). We evaluate these conditions for coexistence in terms of the foraging and cost parameters ($a$, $h$, and $c$). In doing so, we will refer to the cream skimmer (species 1) as the species with a higher positively valued profit than the crumb picker (species 2) at high values of resource availability, and the crumb picker as vice-versa. Thus, the crumb picker has the lower $R^*$. In the absence of such a trade-off, yet again, the species with the lowest $R^*$ would outcompete the other.

A trade-off between encounter rate and handling time can fulfill the assumptions for coexistence on a pulsed resource. The cream skimmer has the lower values for handling time and encounter rate than the crumb picker: $a_1 < a_2$ and $h_1 < h_2$. We will assume that they share the same values for conversion efficiency, $b$, and cost of existence, $c$. So long as consuming the resource is profitable for both ($1 > ch_i$), then: (1) the crumb picker has the lower $R^*$, (2) the cream skimmer has a higher net profit rate than the crumb picker at high values of $R$ (as $R$ gets very large, a consumer's harvest rate converges on $1/h$, and $1/h_1 > 1/h_2$), and (3) the crumb picker has a higher net profit rate than the cream skimmer at low values of $R$ (as $R$ gets very low, a consumer's harvest rate converges on $a$, and $a_2 > a_1$) (**Figure 1A**). Thus there will exist a unique $R'$ where both consumer species have the same net profit rate:

$$R' = \frac{a_2 - a_1}{a_1 a_2 (h_2 - h_1)}$$

We assume that every T time units there occurs a new pulse of resources that achieves a concentration of $R_0$. The consumers can deplete this resource, but renewal does not occur until the next pulse which achieves the same level regardless of leftover resources from the prior pulse (**Figure 2B**).

We assume that changes in population sizes of the consumers occur at the end of each pulse based on the integral of their net profit rate over the course of the interval. Thus, resource levels, $R(t)$, change continuously over the interval $t = 0$ to $t = T$, but consumer population sizes do not (semi-discrete consumer-resource model; Pachepsky et al., 2008):

$$\frac{dR}{dt} = -\sum \frac{x_i a_i R(t)}{1 + a_i h_i R(t)}, \text{ where } R(0) = R_0$$

$$x_i(T) = x_i(0) * e^{b_i \int \left[\frac{a_i R(t)}{1 + a_i h_i R(t)} - c_i\right] dt}$$

There are several immediate results. For coexistence, the initial pulse size $R_0$ must be greater than the consumer species' $R^*$.

To have the lower $R^*$, the crumb picker's $R^*$ must lie in the region where it has a higher profit gain than the cream skimmer: ${R_2}^* < R'$. The cream skimmer's $R^*$ may be greater than or less than $R'$; but for our simulations we shall assume that both species $R^*$'s are less than $R'$ and in the region of $R(t)$ where the crumb picker has the higher net profit rate.

At equilibrium population sizes, the integral of a consumer's net profit rate must be zero. Hence, there must be earlier times during the pulse where resource abundances yield positive profits that are canceled out by later times when resource abundances yield negative profits. Thus, at a single species $x^*$: $R_0 > R^* > R(T)$. This generates an interesting result. The equilibrium population size of a single consumer species must lie along this interval:

$$\frac{R_0}{cT} > \left(x^* = \frac{R_0 - R(T)}{cT}\right) > \frac{R_0 - R^*}{cT}$$

The advantage of this relationship is that $R_0$ is known and $R^*$ can be solved analytically while $R(T)$ cannot be solved for analytically. Additionally, as $R_0$ increases and becomes very large, the leftover resources at the end of the pulse, $R(T)$, decline and converge on $R(T) = 0$ as $R_0$ goes to infinite.

The value of $R_0$ determines the community of consumer species. When $R_0$ is less than the crumb picker's $R^*$, then neither consumer species can exist. Then there is a critical value of $R_0 = R_{12} > {R_2}^*$ below which either the cream skimmer is outcompeted by the crumb picker, or it cannot persist at all. Above this level, $R_{12} > R_0$, the cream skimmer will be present in the community, as it is able to invade a community of crumb pickers at their equilibrium. Then there is another critical value of $R_0 = R_{12} > R_{21}$, below which the crumb picker can invade a community of cream skimmers at their equilibrium and above which the cream skimmers will outcompete the crumb pickers (**Figures 1B,C**). As $R_0$ increases, the equilibrium population size of crumb pickers steadily increases, but as soon as the cream skimmer is present, further increases in $R_0$ lead to an increase in the cream skimmer population size at the expense of the crumb pickers (**Figure 1C**).

We can use simulations to illustrate all of this. We fixed the parameter values of the crumb picker and then used a scaling factor to create a continuum of possible cream skimmer species. To do this, we define a scaling factor $\mu > 1$, where $a_1 = a_2/\mu$ and $h_1 = h_2/\mu$. The divergence between the crumb picker and the cream skimmer increases with $\mu$. As $\mu$ increases, the cream skimmers $R^*$ increases (this will always be true for $\mu > 2$; and for all $\mu > 1$, so long as $ch_2 < 0.5$) and the value of $R'$, the resource abundance where the cream skimmer and crumb picker have the same profit rate, increases. As the cream skimmer becomes more so relative to the crumb picker (increasing $\mu$), both the upper and lower bounds of $R_0$ that produce coexistence increase, even as the region of coexistence expands (**Figure 1B**). **Figure 2** shows an example of coexistence for $\mu = 5$ and an intermediate value of initial pulse size ($R_0 = 30$) (see **Supplementary Figures 1, 2** for examples of population dynamics when coexistence is precluded).

## Key Results

The species with the higher encounter rate, relative to the cost of existence, will have the lower $R^*$ and be the crumb picker, and the one with the lower handling time, relative to the cost of existence, will have the higher $R^*$ and be the cream skimmer. At intermediate values for pulse sizes, coexistence of a crumb picker with a cream skimmer species (or cancer cell type) is expected. At pulse sizes below or above this range, the crumb picker or the cream skimmer should outcompete the other, respectively, thus forming single species communities. As the trade-off in encounter rate and handling time become more extreme, the range of pulse sizes permitting coexistence

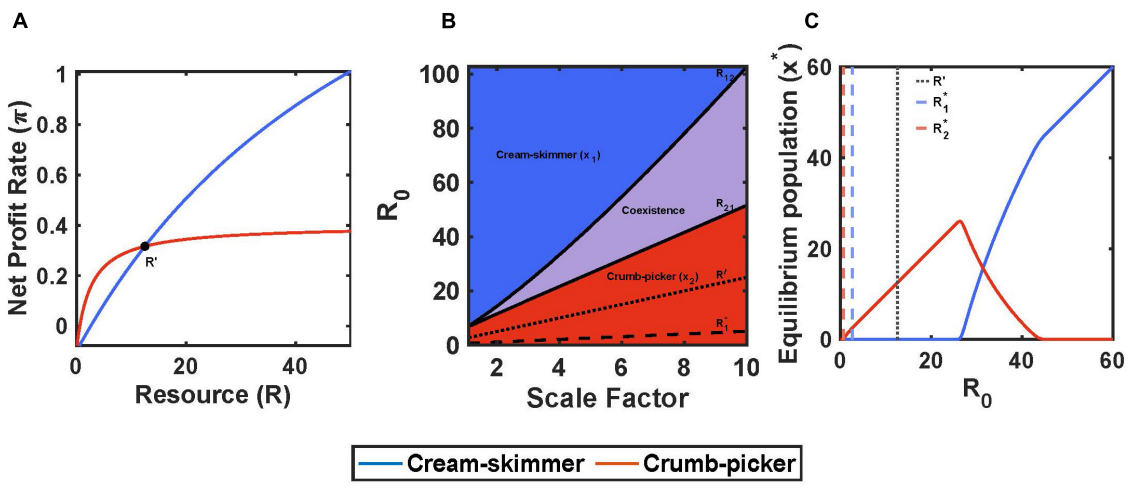


**FIGURE 1 |** Encounter rate and handling time trade-off with pulsed resource. **(A)** The crumb-picker has the higher net profit rate than the cream-skimmer at low values of $R$. $R'$ represents the resource level where both species have the same net profit rate. **(B)** Regions of competitive exclusion and coexistence change due to initial pulse size $R_0$ and the scale factor $\mu$. **(C)** For $\mu = 5$, equilibrium populations change as a function of $R_0$. Intermediate values of $R_0$ show species coexistence. Parameters: $a_2 = 0.2$, $a_1 = a_2/\mu$, $h_2 = 2$, $h_1 = h_2/\mu$, $b_1 = b_2 = 0.5$, $c_1 = 0.1$, $T = 10$, $x_1(0) = 10$, $x_2(0) = 10$.

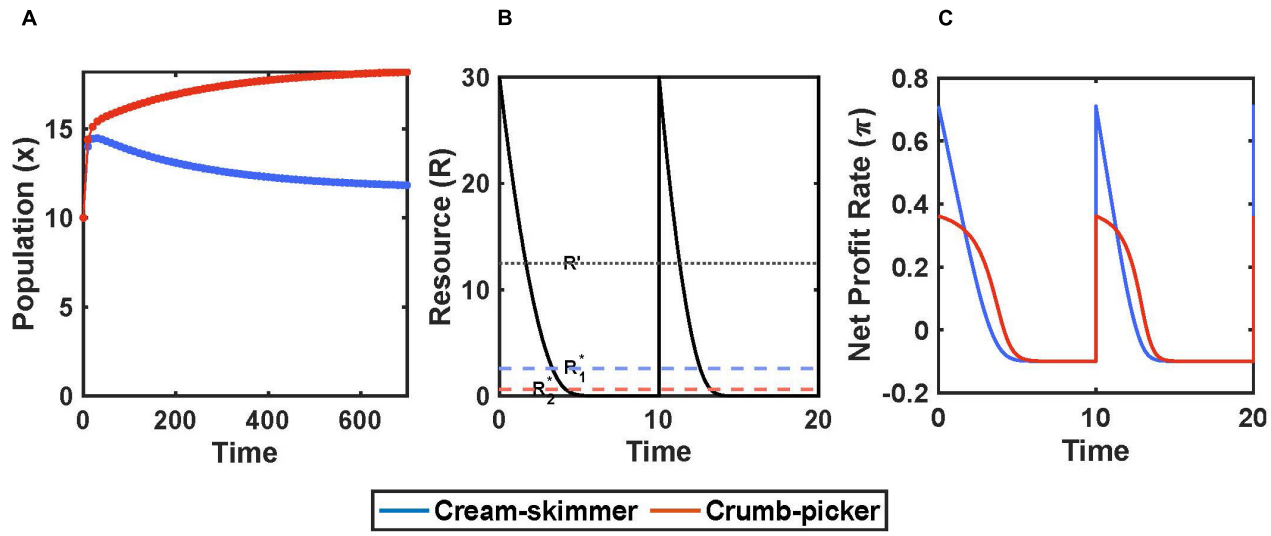


**FIGURE 2 |** Encounter rate and handling time trade-off with pulsed resource. **(A)** Species coexist when $R_0 = 30$ and $\mu = 5$. **(B)** There is a pulse of resource every $T = 10$ units of time. The crumb picker has the lower subsistence level of resource, $R^*_2$. **(C)** Net profit rate of consumers in response to pulse of resource. Parameters: $a_1 = a_2/\mu$, $a_2 = 0.2$, $h_1 = h_2/\mu$, $h_2 = 2$, $b_1 = b_2 = 0.7$, $c_1 = c_2 = 0.1$, $x_1(0) = 10$, $x_2(0) = 10$.

expands, even as the cream skimmer requires higher pulse sizes to be present in the community. This result emerges from how foraging efficiencies change with resource level, $H(R)/c$. As long as $1/(h_1c_1) > 1/(h_2c_2)$, then there will always exist a resource level below which the crumb picker is the more efficient forager (because of $a_2/c_2 > a_1/c_1$) and above which the cream skimmer is the more efficient one.

### Significance

Variability in resource levels can serve as a consumable resource, thus permitting coexistence (Levins, 1979; Armstrong and McGehee, 1980; Chesson, 1994). Here, we place this into a foraging framework where the cream skimmer benefits more from the variance of abundances, while promoting a higher mean level of resources. The crumb picker benefits more from the mean, while promoting a higher variance in temporal resource availabilities. Body size in mammals may represent such a trade-off between cost-adjusted handling times and encounter rates (Brown et al., 2017). In cancer, most cell culture experiments include refreshing the growth medium every 3–6 days, creating regular pulses of resources. The implications of such pulsing have not been investigated for cancer cells' uptake dynamics, competition between difference cell lines, or as a system for testing for cream skimmers and crumb pickers. The functional response curves, $H(R)$, of cancer cells have not been measured. Furthermore, the way a cell line is cultured drives evolution (Burdall et al., 2003). Based on the possibility of an encounter rate versus handing time trade-off, it would be interesting to see whether low resource media (usually in the form of diluted fetal bovine serum) that is changed frequently selects for higher uptake rates at low resource levels at the expense of uptake rates at high levels, and vice-versa for high concentration media changed infrequently.

## Pulsed Resource: Handling Time and Cost of Existence Trade-Off

Cream skimmers and crumb pickers also can be generated from a trade-off between handling time, $h$, and the cost of existence, $c$. Here, we will assume that both species have the same encounter rate with resources, $a$, but that the cream skimmer has a lower handling time and higher cost of existence. This will cause the profit curves as a function of resource abundance to cross at some value of $R$. This happens because the cream skimmer has a lower y-intercept because of a larger cost of existence, yet the cream skimmer has a higher maximum profit by virtue of the lower handling time (**Figure 3A**).

When setting the net profit rate of the crumb picker equal to that of the cream skimmer to solve for $R'$, one finds a more complicated relationship than for the case of $a$ versus $h$. The solution is quadratic on $R'$ (see **Supplementary Material**). One solution will always involve negative values for both $R'$ and the net profit rate. The other solution is relevant and involves a positive value for $R'$, though at $R'$ the net profit rate may be positive or negative depending upon the magnitudes of $a$, $h_1$, $h_2$, $c_1$, and $c_2$.

If the positive solution for $R'$ is greater than the crumb pickers $R_2{}^*$ and the foraging efficiency of the cream skimmer is higher at some level of resource ($1/(h_1c_1) > 1/(h_2c_2)$), then there will exist an initial pulse size $R_{21}$ below which the crumb picker will outcompete the cream skimmer and above which there will be coexistence. Furthermore, there will also be an $R_{12}$ below which coexistence occurs and above which the cream skimmer outcompetes the crumb picker (**Figures 3B,C**). Coexistence will occur when the initial pulse size falls between these two values: $R_{21} < R_0 < R_{12}$.

To illustrate these outcomes we scaled the trade-off using a scaling factor $\mu > 1$ where $h_1 = h_2/\mu$ and $c_1 = c_2 + 0.025\ \mu$.

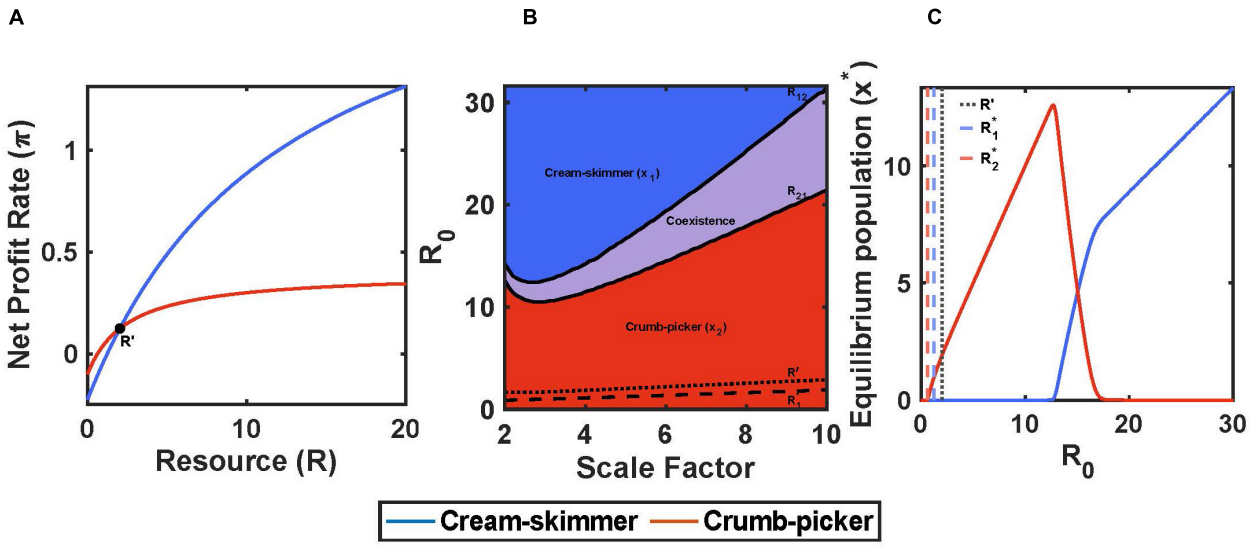


**FIGURE 3 |** Handling time and cost of existence trade-off with pulsed resource. **(A)** The crumb-picker has the higher net profit rate than the cream-skimmer at low values of $R$. $R'$ represents the resource level where both species have the same net profit rate. **(B)** Regions of competitive exclusion and coexistence change due to initial pulse size $R_0$ and the scale factor $\mu$. **(C)** For $\mu = 5$, equilibrium populations change as a function of $R_0$. Intermediate values of $R_0$ show species coexistence. Parameters: $\mu = 5$, $h_2 = 2$, $h_1 = h_2/\mu$, $c_2 = 0.1$, $c_1 = c_2$+(0.025*$\mu$), $b_1 = b_2 = 0.5$, $a_1 = a_2 = 0.2$, $T = 10$, $R_0 = 10$, $x_1(0) = 10$, $x_2(0) = 10$.

The values for $R_{21}$ and $R_{12}$ at first decline and then increase with the magnitude of the trade-off, $\mu$. Regardless, the region of coexistence increases with the magnitude of the trade-off (**Figure 3B**). Otherwise the patterns of coexistence resemble closely those for the $a$ versus $h$ trade-off.

### Key Results

Like the $a$ versus $h$ trade-off, an $h$ versus $c$ trade-off provides the necessary conditions for coexistence on a pulsed resource. While the cream skimmer always has a higher harvest rate than the crumb picker for all resource abundances, its higher cost of foraging drives the intersection of the two species profit curves with resource abundance. The actual conditions require: $h_1 < h_2$; $c_1 > c_2$ and $(1/h_1 - c_1) > (1/h_2 - c_2)$ where this last term is the asymptotic maximum profit as the initial pulse size becomes very large. As the more efficient forager, the crumb picker can always achieve a higher equilibrium population size when alone than can the cream skimmer species. This trade-off represents a foraging speed versus foraging efficiency trade-off.

### Significance

Speed versus efficiency trade-offs are ubiquitous in nature. They can involve different taxa such as reptiles versus mammals, or strategies of plants of more xeric versus more mesic conditions, including varying water use efficiencies (Miller-Rushing et al., 2009; Lanning et al., 2020). An intriguing possibility may be coexisting pinon pine and juniper (Limousin et al., 2015). The pine has more roots that extend less far and less deep (Schwinning et al., 2020) and respond quickly to short pulses of summer rain (West et al., 2007). Thus the pinon pine (cream skimmer) may have a lower overall encounter rate with water, but a rapid and efficient means for handling water and nutrient uptake. A speed versus efficiency trade-off may be particularly relevant to cancer cells in the context of the Warburg effect (Gillies and Gatenby, 2007; Bhattacharya et al., 2016). Cells showing this effect maintain anaerobic glycolysis even in the presence of oxygen. Anaerobic glycolysis permits rapid, yet inefficient, use of glucose; whereas oxidative phosphorylation, through mitochondria, represents a slower but more efficient use of glucose (Epstein et al., 2017).

## Pulsed Resource: Encounter Rate Versus Cost of Existence Trade-Off

A trade-off between encounter rate, $a$, and cost of existence, $c$, provides similar opportunities for coexistence as do the $a$ versus $h$ or $h$ versus $c$ trade-offs. But, coexistence requires that there be a positive handling time: $h > 0$.

With $h = 0$, the species with the lowest $R^*$ will outcompete the other, regardless of the initial pulse size, $R_0$. This is because relative foraging efficiency is now independent of resource abundance. It is everywhere given by $a/c$. The curves of net profit rate, $\pi$, versus resource abundance, $R$, are straight lines with $y$-intercepts of $-c$, $x$-intercepts of $R^*$ and slopes of encounter rate $a$. The species with the higher a/c, has the lower $R^*$ and it will always outcompete the other (**Supplementary Figure 3**).

When $h > 0$ and equal between the two species, a trade-off between $a$ and $c$ can promote coexistence. The species with the higher $a$ must have a higher $c$ that is not proportionately larger than it's $a$ relative to the other species. Furthermore, the species with the higher encounter rate and higher cost of existence (all relative to the fixed $h$) is in fact the crumb picker (species 2): $a_1 < a_2$, $c_1 < c_2$. Coexistence between the two species is possible if $R_1^* > R_2^*$, and $a_2/a_1 > c_2/c_1 > 0$. With these conditions, the crumb picker has the higher foraging efficiency at very low

resource abundances ($a_2/c_2 > a_1/c_1$), and the cream skimmer at high ($1/(h_1c_1) > 1/(h_2c_2)$). Under these conditions, the profit curves with resource abundance are non-linear, have $y$-intercepts at $-c$, $x$-intercepts at $R^*$, and asymptotes at $(1/h - c)$ (**Figure 4A**; see **Supplementary Material**).

For the coexistence of the cream skimmer with the crumb picker, the species' profit curves intersect twice at positive values of $R$. At very low values of $R$ the cream skimmer actually has the higher profit. Beyond the first intersection point, $R'_1$, the crumb picker now has the higher profit, though at this intersection point both species experience negative profits. At a still higher value of $R$, there is the second intersection point, $R'_2$, above which the cream skimmer retains a higher profit rate than the crumb picker for all values of $R > R'_2$ (**Figure 4A**). As the initial pulse size increases, there is the region where no consumer species can exist (necrotic zone in cancer; $R_0 < {R_2}^*$), a region where the crumb picker outcompetes the cream skimmer ($R_0 < R_{21}$), a region of coexistence ($R_{21} < R_0 < R_{12}$), and then a region where the cream skimmer outcompetes the crumb picker ($R_0 > R_{12}$). **Figure 4B** shows how the abundances of resources at which communities switch from one to another ($R_{21}$ and $R_{12}$) decline with a scaling factor that makes the difference between the cream skimmer and crumb picker more extreme with respect to $a$ and $c$. Thus, the cream skimmer actually becomes favored at lower and lower pulse sizes as the two species' trade-off becomes more extreme. **Figure 4C** shows how the equilibrium population sizes of crumb pickers and cream skimmers change with $R_0$.

### Key Results

A trade-off between encounter rate and the cost of existence leads to similar opportunities for coexistence as the $a$ versus $h$, and $h$ versus $c$ trade-offs with a twist. When handling times are equal, it is the species with the higher $a$ and $c$ that is the crumb picker. Relative to the cream skimmer, the crumb picker's proportional increase in $a$ must be greater than its proportional increase in $c$. If there is no handling time, then coexistence is not possible. The species with the higher $a/c$ will always outcompete the other.

### Significance

Mechanisms that change encounter rates in plants include stomatal number, root area, and leaf area. For example, the widespread desert perennial sagebrush produces short-lived "extra" leaves in spring, when water pulses into the desert ecosystem. These leaves increase the encounter rates with light and $CO_2$, have high photosynthetic carbon fixation but low water use efficiency, and are shed before water becomes limiting and costly to use (Evans and Black, 1993). A similar potential mechanism for cancer cell types could be the number of glucose transporters (GLUT1) (Younes et al., 1997; Loponte et al., 2019; Kondo et al., 2021). Upregulating more transporters should increase a cancer cell's encounter rate with glucose molecules, while raising the metabolic costs of producing and maintaining these transporters. This mechanism could be further enhanced by (1) changing the functional response, for instance, a Type 3 functional response (Morozov, 2010), where $\gamma_i > 0$:

$$H_i(R) = \frac{a_i R^{\gamma}}{1 + a_i h_i R^{\gamma}}$$

or, (2) making encounter rates dependent on resource abundance, $a_i(R)$, as might occur when foragers develop a search image (Dukas and Kamil, 2001).

Environmental heterogeneity and trade-offs in foraging parameters form the basis for many mechanisms of coexistence. But, not all trade-offs in foraging parameters will result in coexistence (see Vincent et al., 1996). For our model, appropriate trade-offs between any of the three profit parameters can promote coexistence of a cream skimmer and crumb picker on a pulsed resource.

## Pulsed Resource: Trade-Offs Between Fixed and Total Costs

Virtually all organisms, including microbes, incur an additional variable cost, $v$, when actively foraging or taking up nutrients. Being inactive allows the organism to forgo this cost while still incurring some fixed cost of existence, $f$. Thus, we can break the cost of existence into these two components: $c = v + f$. When actively harvesting resources, the consumer expends both the fixed and variable costs; but, if the consumer so chooses, it can rest. When resting it harvests no resources but only expends the fixed cost. If the consumer's harvest rate is less than the variable cost of foraging, it would be best to rest. Hence, be active when $H(R) > v$ and remain inactive when $H(R) < v$:

$$\frac{d\pi_i}{dt} = \frac{a_i R(t)}{1 + a_i h_i R(t)} - c_i, \text{ when foraging}$$

$$\frac{d\pi_i}{dt} = -f_i, \text{ when resting}$$

where $\pi$ is the consumer's net profit rate.

In the prior models all costs were fixed costs ($v = 0$) and so the consumers never rested. With a pulsed resource and a variable cost greater than zero, $v > 0$, there is now a switch density, $R_s$, where the forager should become inactive when $R(t) < R_s$. This switch can be found by setting the change in profit from foraging equal to that when resting:

$$R_{is} = \frac{v_i}{a_i\left(1 - v_i h_i\right)}$$

Note that $R_s < R^*$ for $f > 0$, meaning that consumers will switch to resting at a resource abundance less than their subsistence level. It pays to operate at a loss of profit so long as the harvest rate covers the variable cost of foraging.

Cancer cells are known to have quiescent states that can include cell cycle arrest, reduced nutrient uptake, and reduced metabolic expenditures (Valcourt et al., 2012; Miller et al., 2021). While quiescence can be induced by nutrient deprivation, it remains an open research question whether cancer cells behaviorally shift from active feeding to a non-feeding resting state in response to the profitability of each activity (White et al., 2020). If they do, a pulsed resource with a trade-off between fixed costs and total costs, $f$ versus $c$, can promote the coexistence of a cream skimmer and crumb picker.

For this model, we will assume that encounter rates and handling times are the same for both consumer species. We

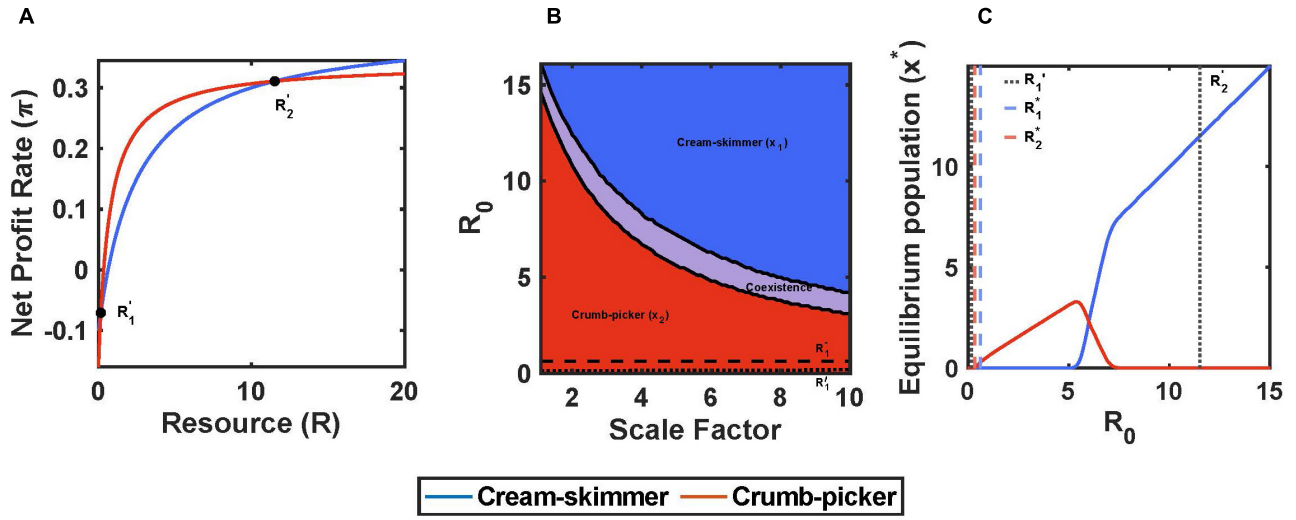


**FIGURE 4 |** Encounter rate and cost of existence trade-off with pulsed resource, $h_1 = h_2 = 2$. **(A)** The crumb-picker has the higher net profit rate than the cream-skimmer for $R'_1 < R < R'_2$. **(B)** Regions of competitive exclusion and coexistence change due to initial pulse size $R_0$ and the scale factor $\mu$. **(C)** For $\mu = 5$, equilibrium populations change as a function of $R_0$. Intermediate values of $R_0$ show species coexistence. Parameters: $\mu = 5$, $a_1 = 0.2$, $a_2 = 0.1$ $\mu$+$a_1$, $c_1 = 0.1$, $c_2 = 0.012$ $\mu$+$c_1$, $b_1 = b_2 = 0.5$, $T = 10$, $R_0 = 10$, $x_1(0) = 10$, $x_2(0) = 10$.

identify the cream skimmer as the species with the higher total cost of existence, $c$, the lower fixed cost, $f$, and the higher $R^*$. For simplicity, we assume that the crumb picker has no variable cost and hence remains active all of the time; its switch density is $R_{2s} = 0$. Under these circumstances, the profit curve of the crumb picker as a function of resource availability has a y-intercept of $-c_2$, an x-intercept of $R_2{}^*$, and a profit rate at maximum harvest rate of $(1/h_2 - c_2)$. The cream skimmer's profit curve has a discontinuity at $R_{1s}$. Below this switch value, profit as a function of $R$ is simply $-f_1$ because it is resting. Above this value, the cream skimmer is active and has the same curve as the crumb picker's, but shifted downwards by $c_1 - c_2$, with an x-intercept of $R_1{}^*$ and a maximum profit rate of $(1/h_1 - c_1)$. The intersection of the crumb picker's and cream skimmer's profit curves, $R'$, occurs at a value less than the cream skimmer's switch density and in the region of negative profit gain (**Figure 5A**).

As shown in the simulations, the success of the crumb picker can be insured so long as $R_{1s} > R_2{}^*$. If the cream skimmers become inactive at a level of resource at which the crumb pickers still make a positive profit, then there are always profitable resources to be had, no matter the population size of cream skimmers or the initial pulse size (so long as $R_0 > R_2{}^*$). In the truest sense, the cream skimmers leave "crumbs" that are valuable to the crumb picker. Thus, there is no pulse size above which the cream skimmers can competitively exclude the crumb pickers. There still remains a pulse size, $R_{21}$, above which the cream skimmers will be present and coexist with the crumb pickers (**Figure 5B**). Above the point where the cream skimmers can join a community of crumb pickers, the population size of cream skimmers increases rapidly with pulse size, as that of the crumb pickers declines to a positive asymptote determined by how many crumb pickers can be supported from effectively having just $R_{1s}$ to work with (**Figure 5C**). The cream skimmers succeed because their low fixed cost allows them to travel inexpensively through time from the point of too few resources to the next resource pulse. If we let $t_{1s}$ be the time at which the cream skimmers switch from foraging to resting during the intra-pulse period, we see that it takes fewer resources to support a cream skimmer than a crumb picker. In the region of coexistence, $c_1 t_{1s} + (T - t_{1s}) f_1 < c_2 T$.

## Key Points

Coexistence of a cream skimmer and crumb picker becomes highly likely on a pulsed or seasonal resource when (1) foragers can choose to be active or to rest or remain dormant, and when there is a trade-off between maintenance efficiency ($H/f$ = efficiency of traveling through time from one good period to the next) and (2) foraging efficiency ($H/c$ = ability to profitably forage resources to a low level). This applies where the forager will switch from foraging to some form of resting when resource abundances have become sufficiently depleted.

## Significance

In nature, this ubiquitous mechanism of coexistence can apply to annual or ephemeral plants (cream skimmers) versus perennials (crumb pickers) (Brown, 1989a), hummingbirds and bees (Brown et al., 1981), colonial bees versus solitary bees (Schaffer et al., 1979), mosses (McNickle et al., 2016), phytoplankton (Litchman and Klausmeier, 2001), and more, and can extend to more species along resource continua (e.g., Chesson et al., 2004, 2013; Angert et al., 2009). While the conditions for this mechanism appear to be met in cancer, it has not been tested or verified. As discussed, the trade-off between anaerobic and aerobic metabolism may allow a Warburg phenotype to coexist with cancer cells that have near-normal metabolism. While expensive, much of the machinery for glycolysis is variable cost and can be

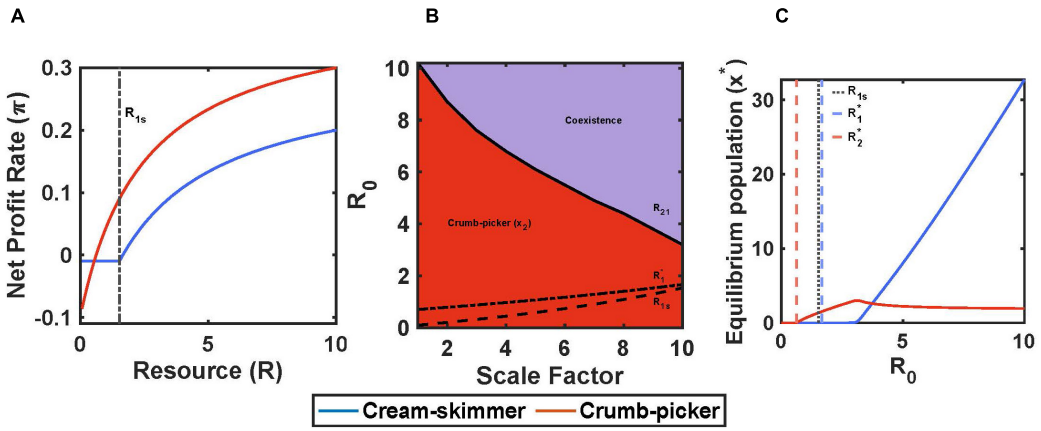


**FIGURE 5 |** Fixed and total costs trade-off with pulsed resource. **(A)** The cream-skimmer's net profit rate has a discontinuity at $R_{1s}$: For $R < R_{1s}$ the cream-skimmer is resting, for $R > R_{1s}$ the cream-skimmer is active. **(B)** Regions of competitive exclusion and coexistence change due to initial pulse size $R_0$ and the scale factor $\mu$. **(C)** For $\mu = 10$, equilibrium populations change as a function of $R_0$. Parameters: $\mu = 5$, $f_1 = 0.1$–$0.009^*\mu$, $f_2 = 0.1$, $c_1 = 0.1+0.01^*\mu$, $c_2 = 0.1$, $b_1 = b2 = 0.5$, $a_1 = a_2 = 0.2$, $h_1 = h_2 = 2$, $v_1 = c_1$-$f_1$, $v_2 = c_2$-$f_2$, $T = 10$, $R_0 = 10$, $x_1(0) = 10$, $x_2(0) = 10$.

down-regulated, while the maintenance of mitochondria entails a high fixed cost. Such a trade-off may manifest between two of the most studied breast cancer cell lines, MDA-MB-231 (elevated glycolysis) and MCF-7 (normal aerobic metabolism). Furthermore, cancer cell dormancy has been documented for many cancer types and can provide the basis for this mechanism of coexistence (Miller et al., 2020, 2021).

Freischel et al. (2021), in Gause-style competition experiments using 3-D spheroid cell cultures, found that MDA-MB-231 cells had a stronger competitive effect on MCF-7 cells than vice-versa, even as MCF-7 had the higher intrinsic growth rates and carrying capacities. As cream skimmers, MDA-MB-231 may have a harvest rate advantage at high resource levels (either through a higher $a$ or lower $h$), have a lower foraging efficiency (higher $c$), and lower fixed cost (lower $f$) as compared to the MCF-7 cells. In these cell cultures, the medium was changed every 4 days, providing a new pulse of resources. Such a system holds much promise for testing for coexistence on a pulsed resource. However, at present we do not know each cancer cell line's profit curves with resource abundance, whether they cease activity when resources become scarce, or how quickly and thoroughly they depress the resources of the culture medium prior to the next pulse.

## Cream Skimmers and Crumb Pickers in Cancer: How and Where to Look?

Tumor heterogeneity, both in micro-environmental conditions and in the genetic and phenotypic composition of the cancer cells themselves, is the norm. Such heterogeneities increase with tumor growth and disease spread. Tumor heterogeneity is generally, though not always (Yu et al., 2017), associated with a poor prognosis for the patient. Histologies with immunohistochemical staining provide one method for identifying cancer cell phenotypes and identifying the diversity of "Darwin's finches" comprising the community of coexisting cancer cell types.

Our model applies to circumstances, likely in tumors, where the pulsing and depletion of resources occurs on a faster time scale than the population dynamics of the consumers. Small scale fluctuations in blood flow, oxygen levels, pH and nutrient supplies give rise to heterogeneity in the microenvironment (Gillies et al., 2018). These temporal variabilities can be stochastic or cyclic (Cárdenas-Navia et al., 2008; Dewhirst, 2009). Scale also plays a role in how nutrient fluctuations occur in the tumor. Recently, Pressley et al. (2021) found approximately 4- to 5-min cycles of $O_2$ levels (and presumably the levels of other nutrients) at small spatial scales within a pancreatic cancer cell line subcutaneously implanted into mice. These results indicate that pulsation and fluctuations of nutrients, the first condition for the coexistence of cream skimmers with crumb pickers, is met in many if not all solid tumors. Furthermore, these changes happen at time scales faster than cancer cell generation times, motivating the use of our semi-discrete consumer-resource model.

The geno- and phenotypic heterogeneity of cancer cells also indicate the potential presence of "cream-skimmer" and "crumb-picker" like cancer cells. Cancer cells expressing hormone receptors are easily characterized with histology. In breast and prostate cancers, estrogen positive or testosterone positive cancer cells require their respective hormones for survival and growth. The frequent coexistence of estrogen positive or testosterone positive cancer cells with estrogen negative or testosterone negative ones that do not require consumption of estrogen or testosterone, respectively, represents a fairly clear case of diet choice (Kareva and Brown, 2021). Furthermore, the different composition of breast cancer cells near vasculature versus away represents spatial separation akin to mesic versus xeric habitats and their associated plant communities (Alfarouk et al., 2013). Finally, coexistence of cell types based on food-safety trade-offs manifest in the different cell types associated with "hot" and "cold" regions of tumors based on high and low immune cell infiltration, respectively (Shembrey et al., 2019; Gatenbee et al., 2020).

Non-hormonal cancers also display differing bio-markers distinguishable in histological studies using a variety of nutrient receptors and metabolic markers. Commander et al. (2020) found that clusters of tumor cells could be divided into leader and follower cells. These two phenotypes displayed distinct metabolic phenotypes. What they referred to as leader cells relied heavily on oxidative phosphorylation with decreased glucose uptake. Conversely, the follower cells relied on glycolysis and required high glucose uptake. This difference could be identified using staining for the glucose transporter, GLUT1, where cream skimmers and crumb pickers would have high and low expression levels, respectively.

Hypoxia markers such as HIF1-α, CAIX, and CAXII also show intra-tumor variation between cancer cells and provide valuable identifiers of cancer stage and prognosis (Chen et al., 2010, 2018; Ilie et al., 2011; Rademakers et al., 2011). Collective production of HIF1-α can promote angiogenesis and increased blood flow to the microenvironment (Yang et al., 2013). To the individual cancer cells it also permits survival and metabolic activity under hypoxic conditions (Kaidi et al., 2007). CAXII is a transmembrane protein often over-expressed in cancers and associated with buffering intra-cellular pH and also permitting survival and activity at low oxygen and nutrient levels (Chiche et al., 2009). We hypothesize that high expression of HIF1-α or CAXII may identify crumb pickers, and at the very least be indirectly associated with our models' foraging parameters.

Diversity of cancer cell metabolism, indicated by upregulated glycolysis (cream skimmer?) or upregulated oxidative phosphorylation (crumb picker?), suggests a speed versus efficiency trade-off. The Warburg effect is likely characteristic of cream-skimmers. These cells maintain high levels of glycolysis (anaerobic respiration) even in the presence of oxygen. In addition to lowering pH, such a strategy increases nutrient uptake, and decreases handling time; but produces much less ATP per respired glucose molecule. Such a strategy entails a low fixed but high variable cost of foraging relative to aerobic respiration via the mitochondria. The transmembrane protein CAIX can provide a marker for cells with upregulated glycolysis (Mboge et al., 2019). CAIX protects against extracellular low pH by creating a protective buffer around the cell, and reducing intra-cellular stress caused by the toxic metabolites from glycolysis. It also may play a role in immune evasion and also represent a food safety trade-off (Lloyd et al., 2016). Upregulated CAIX may provide a biomarker of a cream skimmer strategy.

Genomic analyses (whole genome sequencing for mutations or RNAseq for gene expression) can also identify cancer cell types. Neftel et al. (2019) found four identifiable types of brain cancer cells (glioblastoma). While the frequency of the four types varied with patient and tumor; within a tumor these cell types could be found coexisting in close proximity. One type exhibited traits that were mesenchymal (high motility) and highly glycolytic. This could be a cream skimmer. Any of the other three types might, with further investigation, fit a crumb picker strategy with a slower rate of nutrient uptake and use, but at a lower cost. Sasmita et al. (2018) provide an extensive review of biomarkers and classification schemes for the different subtypes of glioblastomas between patients and of the cancer cell types coexisting within a patient's tumor. The recognized mesenchymal cell type, and the proneural and neural subtypes of cells, may correspond to cream skimmers and crumb pickers, respectively (Verhaak et al., 2010).

## Data From Cancer Patients

We used histologies from 10 breast cancer patients that had been previously stained and scored in Lloyd et al. (2016). Here, we are interested in whether cells with low and high expression of GLUT1, HIF1-α, CAIX or CAXII can be found coexisting in close proximity (unfortunately, the data do not permit examination of how an individual cell scores simultaneously on all four of these stains). **Figure 6** shows an entire biopsy slide for one of the patients and how it can be imaged to highlight the whereabouts of cancer cells. For each of the stains, we identified a subsample in a region with numerous cancer cells. From this cancerous region we created a smaller quadrat 150 μm on a side. For each stain, we found coexisting cancer cells with high and low expression occurring side by side at this small spatial scale (**Figure 7**). For CAIX, CAXII, GLUT1, and HIF1-α, their respective quadrats had **27** versus 20 (**57%**), 22 versus **38** (**33%**), **42** versus 73 (**37%**), and 11 versus **91** (**89%**) cancer cells showing high versus low expression (the bolded numbers and % occurrence are the putative cream skimmers). This pattern of coexistence was manifest across most patients.

We reexamined Lloyd et al.'s (2016) data for frequencies of cell types based on biomarker expression. These data generally provide 60 500 × 500 μm sample quadrats (10 patients × edge versus interior habitats of the tumor × 3 replicates per habitat). CAIX and CAXII data are only available for 9 patients and thus 54 quadrats. The percentages of cells with high expression of the stain within a sample are shown in **Table 2**. High-expressing CAIX cells, perhaps indicative of cream skimmers, were virtually absent (<5%), rare (5–10%), and common in 18, 8, and 1, respectively, of the 27 quadrats at the interior of the tumor (low resources), while 0, 2, and 25, respectively, at the edge of the tumor (high resources) (**Supplementary Table 1**). Low expressing CAIX cells always comprised at least 10% of the cancer cell population. Low CAXII expressing cells, perhaps indicative of cream skimmers, always comprised > 10% of the cancer cell populations both in the interior and edge of the tumors (**Supplementary Table 2**). High expressing CAXII cells (crumb pickers?) always comprised > 10% of cancer cells in the tumors' interiors, but at the edge were <1% in 2 samples, between 1 and 5% in 7 samples, and 5–10% in 6 samples. For GLUT1, high expression (cream skimmers?) was prevalent at the edge of the tumor, but not the interior (**Supplementary Table 3**). At the edge, of the 30 samples, 7 had < 5%, and 5 samples had between 5 and 10%. In the interior, of the 30 samples, 9 had < 1%, 9 between 1 and 5%, 8 between 5 and 10%, and only 4 > 10%. For HIF1α, low expression cells (cream skimmers) were always > 10% of the cells for all samples irrespective of habitat (**Supplementary Table 4**). High expression cells (crumb pickers?) were more common in the interior than edge of the tumor. In the interior, they comprised < 5% in 3 samples, between 5 and 10% in 5 samples, and > 10% in 22 samples. At the edge, they comprised < 5% in 5 samples, between 5 and 10% in 10 samples, and > 10% in 15 samples. Besides opportunities for coexistence of

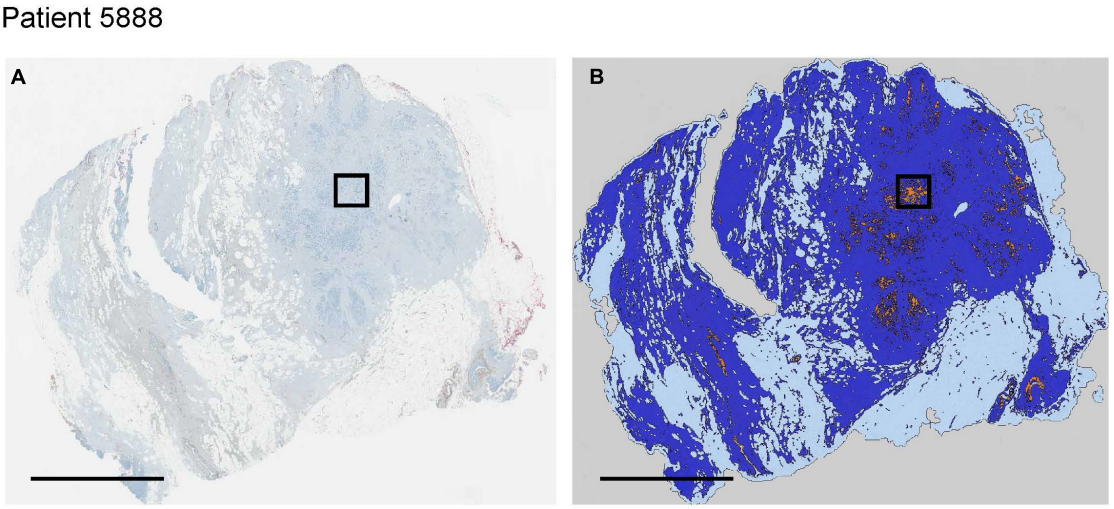


**FIGURE 6 |** For a single invasive breast cancer patient **(A)**, image analysis techniques we used to segment and classify cancer (orange), normal breast tissue (dark blue) and normal adipose tissue (light blue) regions of interest **(B)**. Black bounding box represents the region of interest evaluated in more detail in **Figure 7**. All scale bars = 5 mm.

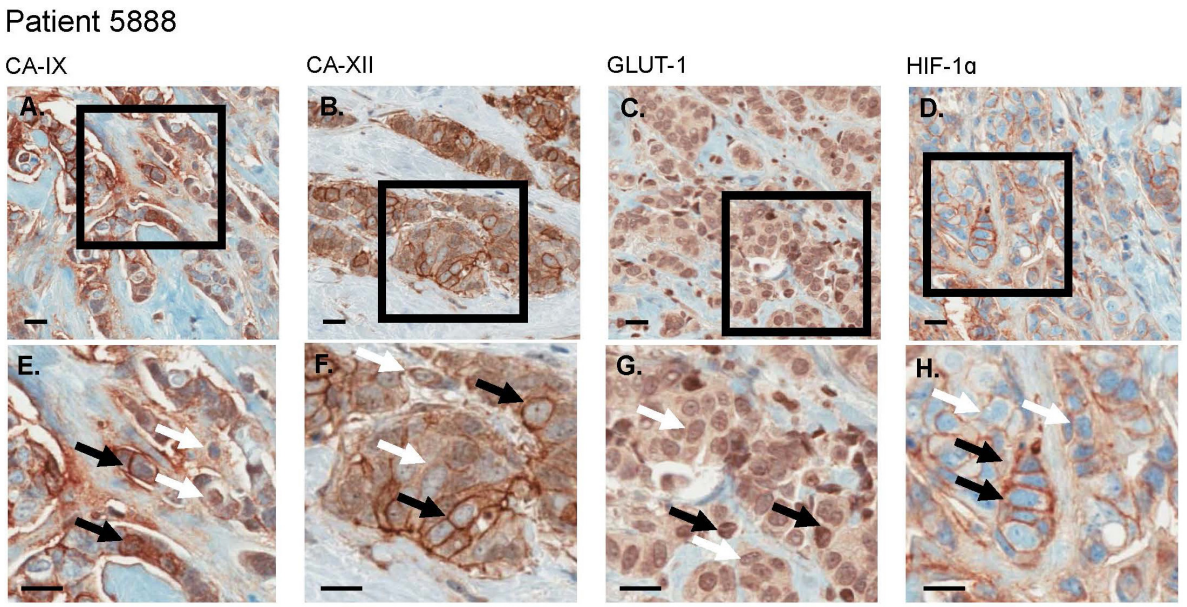


**FIGURE 7 |** For an invasive breast cancer patient, CA-IX, CA-XII, Glut-1, and HIF-1α each demonstrate regions of variable biomarker expression. **(A–D)** display a larger number of cells wherein **(E–H)** are expanded views of selected regions of interest (black bounding boxes). **(E–H)** demonstrate both high (black arrows) and low (white arrows) expression levels (brown stain) in co-mingled populations of cancer cells. All scale bars = 20 μm.

cream skimmers and crumb pickers within specific regions of the tumor, the results also speak to the importance of spatial variation in habitats within tumors in promoting cancer cell heterogeneity (Hoefflin et al., 2016).

For each of these biomarkers there are significant patient-to-patient variation and significant differences between habitats (edge and interior) as originally noted by Lloyd et al. (2016). Furthermore, if any of these biomarkers are indicative of cream skimmers and crumb pickers, then patients, habitats or samples may exhibit examples of just one or the other type, and, in most cases, the coexistence of both types.

As a caveat, note that such histology data might confound true differences in cell types because of overlaps between stain expression and natural variation in the cell's expression that might vary between patients and between tumor habitats within a patient. Furthermore, differences in staining between cells might represent phenotypic plasticity rather than heritable differences. Regardless, we do see strong differences between cancer cells in

**TABLE 2 |** Average percent positive expression of GLUT-1, CAIX, HIF-1α, and CAXII in the center and edge of the tumor.

| Patient | GLUT-1 | | CAIX | | HIF-1α | | CAXII | |
|---|---|---|---|---|---|---|---|---|
| number | Center | Edge | Center | Edge | Center | Edge | Center | Edge |
| 1 | 0.21 | 1.84 | 2.23 | 15.20 | 11.42 | 7.82 | 43.88 | 23.14 |
| 2 | 8.35 | 40.38 | 4.50 | 25.40 | 20.44 | 11.99 | 26.48 | 8.86 |
| 3 | 0.80 | 14.45 | N/A | N/A | 3.11 | 1.38 | N/A | N/A |
| 4 | 5.90 | 5.50 | 1.74 | 11.72 | 7.73 | 8.09 | 49.96 | 12.55 |
| 5 | 1.55 | 4.30 | 5.25 | 18.06 | 19.31 | 17.31 | 32.44 | 3.44 |
| 6 | 5.25 | 44.30 | 5.11 | 16.42 | 26.63 | 21.13 | 50.66 | 13.86 |
| 7 | 6.05 | 32.44 | 6.59 | 16.73 | 22.35 | 4.61 | 45.09 | 4.76 |
| 8 | 0.52 | 21.87 | 2.37 | 11.34 | 9.22 | 7.13 | 41.34 | 5.50 |
| 9 | 23.81 | 6.11 | 9.56 | 23.29 | 27.67 | 21.94 | 47.25 | 25.60 |
| 10 | 4.24 | 23.94 | 3.20 | 28.93 | 38.70 | 36.46 | 22.61 | 0.84 |

*Averages were calculated from six regions of interest (ROI), three from the edge and three from the center of the tumor sample.*

these stains, with many having near to complete absence and others showing very strong expression. And, the mechanism of coexistence can serve to explain coexisting phenotypes whether the basis is plasticity or inheritance.

## DISCUSSION

Temporal variation in resource abundance, or in environmental conditions that affect availability of resources to a consumer, can provide a mechanism of coexistence. These may accompany the more familiar ones of coexisting species partitioning different resources or habitats. Here, we developed and operationalized a model of coexistence on a temporally pulsed resource. We explicitly considered trade-offs in key foraging parameters that can be measured or observed, namely a consumer's encounter rate with resources, $a$ (a measure of foraging speed at low resource abundance), handling time, $h$ (a measure of foraging speed at high resource abundances, where lower is better), and cost of existence, $c$ (a term that determines foraging efficiency at low, $a/c$, versus high, $1/(hc)$, resource abundances). Additionally, we considered the trade-off that can occur when the cost of existence includes a fixed, $f$ (unavoidable cost of being alive), and a variable, $v$ (avoidable if the organism chooses to be inactive), component.

We imagined a pulsed resource, a reasonable proxy to aspects of nature and cancer. Every so many time units, the abundance of resource for the consumers renews to a fixed starting level. Following the pulse, resource abundance declines as consumers consume the resource, and this decline continues until the next pulse. For broad ranges of pulse sizes and trade-offs between two consumer species in foraging parameters ($a$, $h$, and $c$), coexistence is possible between a crumb picker (with the higher $a/c$) and a cream skimmer (with the higher $1/(hc)$). For the fixed and variable cost model, coexistence occurs when the crumb picker has the higher combined foraging efficiency ($H/c$, where $H$ is harvest rate as a function of resource abundance) and the cream skimmer has the higher maintenance efficiency ($H/f$). Both consumer species benefit, when alone, from larger pulse sizes. But, cream skimmers see an increase in their competitive advantage over crumb pickers as the pulse size increases. Throughout, we mention examples from nature around us and suggest putative cancer examples. While well-documented examples exist in the literature on natural ecosystems, the cream-skimmer/crumb-picker trade-off, in its several manifestations, remains untested as a possible explanation for coexisting cancer cell types within a tumor.

Several realistic additional aspects could hamper or facilitate coexistence of a cream skimmer with a crumb picker and might be incorporated into the models we have analyzed here. These include stochastic variation in the timing and sizes of pulses, intra-pulse births and deaths within the consumer species' populations, and some small trickle of resource renewal during the interval following a pulse. A likely important additional factor is the value of a resource item to a consumer, $e$. In effect, we held this constant at unity for both consumers, but there can be trade-offs associated with value. A more complete model would allow for a net profit rate of $eH - c$. Examples of value-dependent net profit can emerge from digestive physiology that create a trade-off between $e$ and $h$ (handling time). For instance, zebra and Canada geese, relative to wildebeest and cottontail rabbits, respectively, are cream skimmers. They do not efficiently digest cellulose. Rather, they consume large amounts of herbage (low $h$), while their digestive system absorbs only a small fraction of the caloric value (low $c$). Wildebeest (ruminants) and cottontails (hindgut fermenters) eat less and take much longer to digest the material (high $h$), but have a higher digestion efficiency (higher $e$). Like the other trade-offs in the consumer-resource model, an $e$ versus $h$ trade-off can promote coexistence if the crumb picker has the higher $ea/c$ and the cream skimmer has the higher $e/hc$. This may be relevant to cancer in that cancer cells with high glycolysis (cream skimmers) versus high oxidative phosphorylation (crumb pickers) may be best represented as having a low $e$ (2 ATPs versus 36 ATPs per glucose molecule) and a low $h$, in addition to other trade-offs associated with $a$, $c$, or $f$.

Cancer may provide a good model for testing the mechanisms we have described. Cancer biologists generally do not measure the key foraging parameters of the consumer-resource model (see Amend et al., 2018; Mallin et al., 2020), but some tools for such measurement are available and can be quite sophisticated. The Seahorse XF Analyzer (de Moura and Van Houten, 2014) can analyze the extracellular flux of a small population or aliquot of cells (normal or cancerous) for oxygen, lactate production, glucose uptake, etc. While used extensively in cancer research, this technique has not yet been used specifically to measure things like a cancer cell's functional response ($H$ versus $R$) in terms of $a$ and $h$. Furthermore, 2-D and 3-D culture experiments are generally run as batch chemostats where the culture medium is removed and replaced every so many days. Careful calibration with respect to cell type, cell numbers, cell proliferation rates, initial resource concentration, ending resource concentration, and pulse frequency could be used to not only estimate model parameters, but also to test for coexistence when competing multiple cell lines (Freischel et al., 2021).

Our modeling results have implications for conducting appropriate cell culture experiments. In general, culture medium,

rich in fetal bovine serum and sometimes augmented with additional resources, is changed every few days. The change is often made with reference to a pH marker to ensure little change in pH via cell metabolites. Furthermore, in 2-D and some 3-D cultures, cells are passaged prior to reaching some level of confluency, meaning they may not reach a true equilibrium with their resource availabilities. As such, we may inadvertently be selecting for cream skimmers or species that speedily but inefficiently turn resources into proliferation. The timing of nutrient pulses and the passaging of cells in culture experiments may be far from what meaningfully occurs in patient tumors or mouse models. Imposing a given pulsing of nutrient renewal may change the whole ecology of the tumor, which may possibly undermine the validity of the interpretation of the results. Our modeling and that of others on consumer resource dynamics invites researchers to be mindful of the ecological conditions of their cell cultures and whether the ecology is realistic or useful for the objectives of the study.

Therefore, it is reasonable to postulate that cell culture experimentation may be used to further elucidate tangible differences in cell survival strategies. Under the lens of live cell, time lapse microscopy, one may observe how resource dynamics affect cell survival strategies and discern if cells are establishing heritable variation versus phenotypic plasticity. Non-invasive live cell microscopy is now possible within incubation conditions which can facilitate multiple generations of cellular growth to confluency, splitting and repeating. Future experiments may be designed to select for cells in resource rich and resource poor environments and observe cellular population growth upon changes in resource allocation after multiple passages.

For cancer, one often is interested in implications of ecological models for cancer therapies. We cannot provide specific recommendations in terms of cancer therapy based on the mechanisms we have described for coexistence of cream skimmer and crumb picker phenotypes. But, more broadly, it is known that tumors that are heterogeneous, including diverse cancer cell types, tend to be associated with worse prognoses. This is generally thought to result from higher levels of heritable variation among the cancer cells and hence a higher likelihood that one or several variants will be resistant to therapy. This may be so. Additionally, therapy failure may result from the consequences of using one or several therapeutic regimens to treat a community of cancer cells, not just a single cancer. If cancer cells are diversifying and filling niches, as in an ecological community, then therapy may be more effective at killing one type of cancer cell and not another. Kotler and Brown (2020) provide thoughts on how therapy strategizing should take into consideration within-patient mechanisms of coexistence among cancer cell types.

With respect to cream skimmers and crumb pickers, a variety of therapies may directly or indirectly influence competitive balance and treatment efficacy. For instance, many chemotherapies target rapidly dividing cells, which may favor crumb-picker strategies among the survivors. Radiation therapy, use of a variety of therapies, and diets that modify the tumor microenvironment (anti-angiogenics, bicarbonate therapies, fasting, ketogenic diets, etc.) may alter the amount and temporal pulsing of nutrients (Gatenby and Brown, 2020). This might harm all cancer cells or might simply tip the competitive scales away from or toward a cream skimmer. Finally, knowing that some of the tumor microenvironment is composed of coexisting cream skimmers and crumb pickers may suggest double-bind therapies (Gatenby et al., 2009; Basanta et al., 2012), where one begins with a therapy that favors one of these types and would lead to competitive exclusion of the other. Upon shifting the cancer community with the first therapy, one then would apply a second therapy to target the remaining and now dominant cancer cell type (Maley et al., 2004).

In this study, we examined temporal variation in resource abundances. Spatial variation can provide an extension of the mechanisms we discussed (Chesson, 2000a). Spatial variation can provide both additional trade-off terms (higher travel speed or lower costs can now define a cream skimmer) and an additional way that the foraging activities of consumers can create temporal variability, particularly if a consumer locally depletes resources faster than they can renew (Richards et al., 2000; Abrams and Wilson, 2004; Bolin et al., 2018). Examples include freshwater snails (Chase et al., 2001), sunbirds (Oyugi et al., 2012), and bees (Aizen et al., 2011). Furthermore, the trade-off between cream skimmers and crumb pickers can include the ability to accurately assess local resource abundances, but at a cost of supporting a higher cognitive ability (Olsson and Brown, 2010). Despite these examples from the ecosystems around us, we anticipate that this spatial form of cream skimmer/crumb picker trade-offs is less likely in cancer, given the limited motility of cancer cells relative to the scale of spatial variation in resources. Even though cancer cells have motile phenotypes (with amoeboid, pseudopodial, and lobopodial movement; Paul et al., 2017; Jun et al., 2020), they do not move very fast and are quite slow compared to free-living unicells such as yeast. Travel speeds in a 3-D collagen matrix were 4.5 μm/h and 2.1 μm/h for a "fast" mesenchymal and "slow" epithelial cancer cell type, respectively. Populations of such cancer cells can have mean diameters of 19–25 μm (Connolly et al., 2020), meaning that it could take at best 4 h to move one body length, and likely much longer. However, we could be wrong about how spatial variation is realized by cancer cells. In reality, biodiversity is affected simultaneously by more than a single mechanism of coexistence, and these operate simultaneously over many temporal and spatial scales; the relative strengths of mechanisms also no doubt vary over time, and which mechanisms are visible will depend on the scales at which we sample and analyze information (Chesson and Huntly, 1993, 1997; Chesson, 2000b, 2009; Chesson et al., 2013; Letten et al., 2018). The cancer ecosystem provides an interesting potential model in which to examine the many temporal and spatial scales and mechanisms that could simultaneously affect coexistence of cream skimmers and crumb pickers.

In conclusion, we think that a mechanism of coexistence of cream skimmers with crumb pickers has broad applicability to all of nature, including cancer. Identifying and studying this mechanism in cancer would provide (1) direct applications and tests of ecological principles in a simpler yet complete ecosystem, (2) applications of consumer-resource models to the diversification of cancer cells within and between patients,

(3) explicit uptake and cost parameters that have not been, but can be, measured for cancer cells, and (4) insights to possible therapeutic implications.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of South Florida Institutional Review Board; Moffitt Scientific Review Committee. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JB and NH conceived the study. AF, AM, and JB analyzed the model and conducted simulations. ML and JB provided and analyzed *in vivo* data. AF, AM, and ML created the figures and tables. JB, NH, and ML wrote portions of the first draft. All authors contributed substantially to revisions, contributed to the article, and approved the submitted version.

## FUNDING

JB gratefully acknowledges funding by NIH/NCI 1U54CA 193489-01A1, "Cancer as a Complex Adaptive System," NIH/NCI U54 Supplement, "The tumor-host evolutionary arms race." DB and AM were partially funded by an NCI PSON U01 CA244101. The data acquisition was sponsored in part by the Moffitt Cancer Center PSOC NIH/NCI U54CA143970. Partial funding was provided by the Utah State University Ecology Center.

## ACKNOWLEDGMENTS

The authors thank Tatiana Miti for discussion and insights on the modeling, Jeffrey West for help with figures, and the Moffitt Tissue Core and the Analytic Microscopy Core for their expertise.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.697618/full#supplementary-material

## REFERENCES

Abrams, P. A. (1984). Variability in resource consumption rates and the coexistence of competing species. *Theor. Popul. Biol.* 25, 106–124. doi: 10.1016/0040-5809(84)90008-x

Abrams, P. A. (2004). When does periodic variation in resource growth allow robust coexistence of competing consumer species. *Ecology* 85, 372–382. doi: 10.1890/02-0684

Abrams, P. A., and Wilson, W. G. (2004). Coexistence of competitors in metacommunities due to spatial variation in resource growth rates; does R* predict the outcome of competition? *Ecol. Lett.* 7, 929–940. doi: 10.1111/j.1461-0248.2004.00644.x

Abrams, P. S., Brassil, C. E., and Holt, R. D. (2003). Dynamics and responses to mortality rates of competing predators undergoing predator-prey cycles. *Theor. Popul. Biol.* 64, 163–176. doi: 10.1016/S00040-5809(03)00067-4

Abrams, P. S., and Holt, R. D. (2002). The impact of consumer-resource cycles on the coexistence of competing consumers. *Theor. Popul. Biol.* 62, 281–295. doi: 10.1006/tpbi.2002.1614

Agrawal, G., Ramesh, A., Aishwarya, P., Sally, J., and Ravi, M. (2021). Devices and techniques used to obtain and analyze three-dimensional cell cultures. *Biotechnol. Progress* 2021:e3126. doi: 10.1002/btpr.3126

Aizen, M. A., Lozada, M., and Morales, C. L. (2011). Comparative nectar-foraging behaviors and efficiencies of an alien and a native bumble bee. *Biol. Invas.* 13, 2901–2909. doi: 10.1007/s10530-011-9972-3

Alfarouk, K. O., Ibrahim, M. E., Gatenby, R. A., and Brown, J. S. (2013). Riparian ecosystems in human cancers. *Evol. Appl.* 6, 46–53. doi: 10.1111/eva.12015

Amaro, A., Chiara, S., and Pfeffer, U. (2016). Molecular evolution of colorectal cancer: from multistep carcinogenesis to the big bang. *Cancer Metastasis Rev.* 35, 63–74. doi: 10.1007/s10555-016-9606-4

Amend, S. R., Gatenby, R. A., Pienta, K. J., and Brown, J. S. (2018). Cancer foraging ecology: diet choice, patch use, and habitat selection of cancer cells. *Curr. Pathobiol. Rep.* 6, 209–218. doi: 10.1007/s40139-018-0185-7

Angert, A. L., Huxman, T. E., Chesson, P., and Venable, D. L. (2009). Functional trade-offs determine species coexistence via the storage effect. *Proc. Nat. Acad. Sci. U.S.A.* 106, 11641–11645. doi: 10.1073/pnas.0904512106

Armstrong, R. A., and McGehee, R. (1980). Competitive exclusion. *Am. Nat.* 115, 151–170.

Armstrong, R. A., and McGehee, R. P. (1976). Coexistence of two competitors on one resource. *J. Theor. Biol.* 56, 499–502. doi: 10.1016/s0022-5193(76)80089-6

Basanta, D., Gatenby, R. A., and Anderson, A. R. (2012). Exploiting evolution to treat drug resistance: combination therapy and the double bind. *Mol. Pharm.* 9, 914–921. doi: 10.1021/mp200458e

Bhattacharya, B., Mohd Omar, M. F., and Soong, R. (2016). The Warburg effect and drug resistance. *Br. J. Pharm.* 173, 970–979. doi: 10.1111/bph.13422

Bolin, A., Smith, H. G., Lonsdorf, E. V., and Olsson, O. (2018). Scale-dependent foraging tradeoff allows competitive coexistence. *Oikos* 127, 1575–1585. doi: 10.1111/oik.05072

Bonsall, M. B., Hassell, M. P., and Asefa, G. (2002). Ecological trade-offs, resource partitioning, and coexistence in a host–parasitoid assemblage. *Ecology* 83, 925–934. doi: 10.2307/3071902

Brown, J. H., Kodric-Brown, A., Whitham, T. G., and Bond, H. W. (1981). Competition between hummingbirds and insects for the nectar of two species of shrubs. *Southwest. Nat.* 26, 133–145. doi: 10.2307/3671109

Brown, J. S. (1989a). Coexistence on a seasonal resource. *Am. Nat.* 133, 168–182. doi: 10.1086/284908

Brown, J. S. (1989b). Desert rodent community structure: a test of four mechanisms of coexistence. *Ecol. Monogr.* 59, 1–20. doi: 10.2307/2937289

Brown, J. S., Kotler, B. P., and Mitchell, W. A. (1994). Foraging theory, patch use, and structure of a Negev Desert granivore community. *Ecology* 75, 2286–2300. doi: 10.2307/1940884

Brown, J. S., Kotler, B. P., and Porter, W. P. (2017). How foraging allometries and resource dynamics could explain Bergmann's rule and the body-size diet relationship in mammals. *Oikos* 126, 224–230.

Burdall, S. E., Hanby, A. M., Lansdown, M. R., and Speirs, V. (2003). Breast cancer cell lines: friend or foe? *Breast Cancer Res.* 5, 1–7.

Cárdenas-Navia, L. I., Mace, D., Richardson, R. A., Wilson, D. F., Shan, S., and Dewhirst, M. W. (2008). The pervasive presence of fluctuating oxygenation

in tumors. *Cancer Res.* 68, 5812–5819. doi: 10.1158/0008-5472.CAN-07-6387

Carvalho, M. R., Lima, D., Reis, R. L., Correlo, V. M., and Oliveira, J. M. (2015). Evaluating biomaterial-and microfluidic-based 3D tumor models. *Trends Biotech.* 33, 667–678. doi: 10.1016/j.tibtech.2015.09.009

Cermeno, P., Lee, J.-B., Wyman, K., Schofield, O., and Falkowski, P. G. (2011). Competitive dynamics in two species of marine phytoplankton under non-equilibrium conditions. *Mar. Ecol. Prog. Ser.* 429, 19–28. doi: 10.3354/meps09088

Chase, J. M., Wilson, W. G., and Richards, S. A. (2001). Foraging trade-offs and resource patchiness: theory and experiments with a freshwater snail community. *Ecol. Lett.* 4, 304–312. doi: 10.1046/j.1461-0248.2001.00216.x

Chen, C.-L., Chu, J.-S., Su, W.-C., Huang, S.-C., and Lee, W.-Y. (2010). Hypoxia and metabolic phenotypes during breast carcinogenesis: expression of HIF-1α, GLUT1, and CAIX. *Virchows Arch.* 457, 53–61. doi: 10.1007/s00428-010-0938-0

Chen, Z., Ai, L., Mboge, M. Y., Tu, C., McKenna, R., Brown, K. D., et al. (2018). Differential expression and function of CAIX and CAXII in breast cancer: a comparison between tumorgraft models and cells. *PLoS One* 13:e0199476. doi: 10.1371/journal.pone.0199476

Chesson, P. (1994). Multispecies competition in variable environments. *Theor. Popul. Biol* 45, 227–276. doi: 10.1006/tpbi.1994.1013

Chesson, P. (2000a). General theory of competitive coexistence in spatially-varying environments. *Theor. Popul. Biol.* 58, 211–237. doi: 10.1006/tpbi.2000.1486

Chesson, P. (2000b). Maintenance of species diversity. *Ann Rev. Ecol. Syst.* 31, 343–366.

Chesson, P. (2009). Scale transition theory with special reference to species coexistence in a variable environment. *J. Biol. Dyn.* 3, 149–163. doi: 10.1080/17513750802585491

Chesson, P., Gebauer, R. L. E., Schwinning, S., Huntly, N., Weigand, K., Ernest, M. S. K., et al. (2004). Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* 141, 236–253. doi: 10.1007/s00442-004-1551-1

Chesson, P., and Huntly, N. (1988). Community consequences of life history traits in a variable environment. *Ann. Zool. Fennici* 25, 5–16.

Chesson, P., and Huntly, N. (1989). How do short-term instabilities affect long-term community dynamics? *Tree* 4, 293–298. doi: 10.1016/0169-5347(89)90024-4

Chesson, P., and Huntly, N. (1993). Temporal hierarchies of variation and the maintenance of diversity. *Plant Species Biol.* 8, 195–206. doi: 10.1111/j.1442-1984.1993.tb00070.x

Chesson, P., and Huntly, N. (1997). The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *Am. Nat.* 150, 519–553. doi: 10.1086/286080

Chesson, P., Huntly, N. J., Roxburgh, S. H., Pantastico-Caldas, M., and Facelli, J. M. (2013). "The storage effect: definitions and tests in two plant communities," in *Temporal Dynamics and Ecological Process*, eds C. Kelly, M. Bowler, and G. Fox (New York, NY: Cambridge University Press), 11–40. doi: 10.1017/cbo9781139048170.003

Chesson, P., and Warner, R. R. (1981). Environmental variability promotes coexistence in lottery competitive systems. *Am. Nat.* 117, 923–943. doi: 10.1086/283778

Chiche, J., Ilc, K., Laferrière, J., Trottier, E., Dayan, F., Mazure, N. M., et al. (2009). Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. *Cancer Res.* 69, 358–368. doi: 10.1158/0008-5472.CAN-08-2470

Commander, R., Wei, C., Sharma, A., Mouw, J. K., Burton, L. J., Summerbell, E., et al. (2020). Subpopulation targeting of pyruvate dehydrogenase and GLUT1 decouples metabolic heterogeneity during collective cancer cell invasion. *Nat. Commun.* 11:1533. doi: 10.1038/s41467-020-15219-7

Connolly, S., McGourty, K., and Newport, D. (2020). The in vitro inertial positions and viability of cells in suspension under different in vivo flow conditions. *Sci. Rep.* 10:1711. doi: 10.1038/s41598-020-58161-w

de Moura, M. B., and Van Houten, B. (2014). "Bioenergetic analysis of intact mammalian cells using the Seahorse XF24 Extracellular Flux analyzer and a luciferase ATP assay," in *Molecular Toxicology Protocols*, eds P. Keohavong and S. G. Grant (Totowa, NJ: Humana Press), 589–602. doi: 10.1007/978-1-62703-739-6_40

Descamps-Julien, B., and Gonzalez, A. (2005). Stable coexistence in a fluctuating environment: an experimental demonstration. *Ecology* 86, 2815–2824. doi: 10.1890/04-1700

Dewhirst, M. W. (2009). Relationships between cycling hypoxia, HIF-1, angiogenesis and oxidative stress. *Radiat. Res.* 172, 653–665. doi: 10.1667/rr1926.1

Di Gregorio, A., Bowling, S., and Rodriguez, T. A. (2016). Cell competition and its role in the regulation of cell fitness from development to cancer. *Dev. Cell* 38, 621–634. doi: 10.1016/j/devcel.2016.08.012

Dukas, R., and Kamil, A. C. (2001). Limited attention: the constraint underlying search image. *Behav. Ecol.* 12, 192–199. doi: 10.1093/beheco/12.2.192

Epstein, T., Gatenby, R. A., and Brown, J. S. (2017). The Warburg effect as an adaptation of cancer cells to rapid fluctuations in energy demand. *PLoS One* 12:e0185085. doi: 10.1371/journal.pone.0185085

Evans, R. D., and Black, R. A. (1993). Growth, photosynthesis, and resource investment for vegetative and reproductive modules of *Artemisia tridentata*. *Ecology* 74, 1516–1528. doi: 10.2307/1940079

Freischel, A. R., Damaghi, M., Cunningham, J. J., Ibrahim-Hashim, A., Gillies, R. J., Gatenby, R. A., et al. (2021). Frequency-dependent interactions determine outcome of competition between breat cancer cell lines. *Sci. Rep.* 11:4908. doi: 10.1038/s41598-021-84406-3

Gatenbee, C. D., Minor, E. S., Slebos, R. J., Chung, C. H., and Anderson, A. R. (2020). Histoecology: applying ecological principles and approaches to describe and predict tumor ecosystem dynamics across space and time. *Cancer Control* 27:1073274820946804.

Gatenby, R. A., Brown, J., and Vincent, T. (2009). Lessons from applied ecology: cancer control using an evolutionary double bind. *Cancer Res.* 69, 7499–7502. doi: 10.1158/0008-5472.can-09-1354

Gatenby, R. A., and Brown, J. S. (2020). Integrating evolutionary dynamics into cancer therapy. *Nat. Rev. Clin. Oncol.* 17, 675–686. doi: 10.1038/s41571-020-0411-1

Gillies, R. J., Brown, J. S., Anderson, A. R. A., and Gatenby, R. A. (2018). Ecoevolutionary causes and consequences of temporal changes in intratumoural blood flow. *Nat. Rev. Cancer* 18, 576–585. doi: 10.1038/s41568-018-0030-7

Gillies, R. J., and Gatenby, R. A. (2007). Adaptive landscapes and emergent phenotypes: why do cancers have high glycolysis? *J. Bioenerg. Biomembr.* 39, 251–257. doi: 10.1007/s10863-9085-y

Grover, J. P. (1990). Resource competition in a variable environment: phytoplankton growing according to Monod's model. *Am. Nat.* 136, 771–789. doi: 10.1086/285131

Hallett, L. M., Shoemaker, L. G., White, K. T., and Suding, K. N. (2019). Rainfall variability maintains grass-forb species coexistence. *Ecol. Lett.* 22, 1658–1667. doi: 10.1111/ele.13341

Hardin, G. (1961). The competitive exclusion principle. *Science* 131, 1292–1297. doi: 10.1126/science.131.3409.1292

Hoefflin, R., Lahrmann, B., Warsow, G., Hübschmann, D., Spath, C., Walter, B., et al. (2016). Spatial niche formation but not malignant progression is a driving force for intratumoural heterogeneity. *Nat. Commun.* 7:ncomms11845.

Holling, C. S. (1959). Some characteristics of simple types of predation and parasitism. *Can. Entom.* 91, 385–398. doi: 10.4039/ent91385-7

Huisman, J., Johansson, A. M., Folmer, E. O., and Weissing, F. J. (2001). Towards a solution of the plankton paradox: the importance of physiology and life history. *Ecol. Lett.* 4, 408–411. doi: 10.1046/j.1461-0248.2001.00256.x

Huisman, J., and Weissing, F. J. (1999). Biodiversity of plankton by species oscillations and chaos. *Nature* 402, 407–410. doi: 10.1038/46540

Huntly, N. (1991). Herbivores and the dynamics of communities and ecosystems. *Ann. Rev. Ecol. Syst.* 22, 477–503. doi: 10.1146/annurev.es.22.110191.002401

Hutchinson, G. E. (1961). The paradox of the plankton. *Am. Nat.* 95, 137–145.

Ilie, M. I., Hofman, V., Ortholan, C., Ammadi, R. E., Bonnetaud, C., Havet, K., et al. (2011). Overexpression of carbonic anhydrase XII in tissues from resectable non-small cell lung cancers is a biomarker of good prognosis. *Int. J. Cancer* 128, 1614–1623. doi: 10.1002/ijc.25491

Iravani, A., Mitchell, C., Akhurst, T., Sandhu, S., Hofman, M. S., and Hicks, R. J. (2021). Molecular imaging of neuroendocrine differentiation of prostate cancer: a case series. *Clin. Genitourin. Cancer* 19, e200–e205. doi: 10.1016/j.dgc.2021.01.008

Jones, M., Mandelik, Y., and Dayan, T. (2001). Coexistence of temporally partitioned spiny mice: roles of habitat structure and foraging behavior. *Ecology* 82, 2164–2176. doi: 10.1890/0012-9658(2001)082[2164:cotpsm]2.0.co;2

Jun, B. H., Guo, T., Libring, S., Chanda, M. K., Paez, J. S., Shinde, A., et al. (2020). Fibronectin-expressing mesenchymal tumor cells promote breast cancer metastasis. *Cancers* 12:2553. doi: 10.3390/cancers12092553

Kaidi, A., Williams, A. C., and Paraskeva, C. (2007). Interaction between β-catenin and HIF-1 promotes cellular adaptation to hypoxia. *Nat. Cell Biol.* 9, 210–217. doi: 10.1038/ncb1534

Kareva, I., and Brown, J. S. (2021). Estrogen as an essential resource and the coexistence of ER+ and ER- cancer cells. *Front. Ecol. Evol.* 9:673082. doi: 10.3389/fevo.2021.673082

Koch, A. L. (1974). Coexistence resulting from an alternation of density dependent and density independent growth. *J. Theor. Biol.* 44, 373–386. doi: 10.1016/0022-5193(74)90168-4

Kondo, H., Ratcliffe, C. D., Hooper, S., Ellis, J., MacRae, J. I., Hennequart, M., et al. (2021). Single-cell resolved imaging reveals intra-tumor heterogeneity in glycolysis, transitions between metabolic states, and their regulatory mechanisms. *Cell Rep.* 34:108750. doi: 10.1016/j/celrep/2021/108750

Kotler, B. P., and Brown, J. S. (1988). Environmental heterogeneity and the coexistence of desert rodents. *Ann. Rev. Ecol. Syst.* 19, 281–307. doi: 10.1146/annurev.es.19.110188.001433

Kotler, B. P., and Brown, J. S. (1990). Rates of seed harvest by two species of gerbilline rodents. *J. Mamm.* 71, 591–596. doi: 10.2307/1381798

Kotler, B. P., and Brown, J. S. (2020). Cancer community ecology. *Cancer Control* 27:1073274820951776. doi: 10.1177/1073274820951776

Kotler, B. P., Brown, J. S., and Subach, A. (1993). Mechanisms of species coexistence of optimal foragers: temporal partitioning by two species of sand dune gerbils. *Oikos* 67, 548–556. doi: 10.2307/3545367

Lanning, M., Wang, L., Benson, M., Zhang, Q., and Novick, K. (2020). Canopy isotopic investigation reveals different water uptake dynamics of maples and oaks. *Phytochemistry* 175:112389. doi: 10.1016/j.phytochem.2020.112389

Letten, A. D., Dhami, M. K., Je, P.-J., and Fukami, T. (2018). Species coexistence through simultaneous fluctuation-dependent mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* 115, 6745–6750. doi: 10.1073/pnas.1801846115

Levins, R. (1979). Coexistence in a variable environment. *Am. Nat.* 114, 765–783. doi: 10.1086/283527

Limousin, J. M., Yepez, E. A., McDowell, N. G., and Pockman, W. T. (2015). Convergence in resource use efficiency across trees with differing hydraulic strategies in response to ecosystem precipitation manipulation. *Func. Ecol.* 29, 1125–1136. doi: 10.1111/1365-2435.12426

Lisan, A., and Langhans, S. A. (2015). Cancer as a channelopathy: ion channels and pumps in tumor development and progression. *Front. Cell. Neurosci.* 9:86. doi: 10.3389/fncel.2015.00086

Litchman, E., and Klausmeier, C. A. (2001). Competition of phytoplankton under fluctuating light. *Am. Nat.* 157, 170–187. doi: 10.2307/3079257

Litchman, E., and Klausmeier, C. A. (2008). Trait-based community ecology of phytoplankton. *Ann. Rev. Ecol. Syst.* 39, 615–639. doi: 10.1146/annurev.ecolsus.39.110707.173549

Lloyd, M. C., Cunningham, J. J., Bui, M. M., Gillies, R. J., Brown, J. S., and Gatenby, R. A. (2016). Darwinian dynamics of intratumoral heterogeneity: not solely random mutations but also variable environmental selection forces. *Cancer Res.* 76, 3136–3144. doi: 10.1158/0008-5472.CAN-15-2962

Lo, Y. H., Karlsson, K., and Kuo, C. J. (2020). Applications of organoids for cancer biology and precision medicine. *Nat. Cancer* 1, 761–773. doi: 10.1038/s43018-020-0102-y

Loponte, S., Lovisa, S., Deem, A. K., Carugo, A., and Viale, A. (2019). The many facets of tumor heterogeneity: is metabolism lagging behind? *Cancers* 11:1574. doi: 10.390/cancers11101574

Maley, C. C., Reid, B. J., and Forrest, S. (2004). Cancer prevention strategies that address the evolutionary dynamics of neoplastic cells: simulating benign cell boosters and selection for chemosensitivity. *Cancer Epidemiol. Prevent. Biomarkers* 13, 1375–1384.

Mallin, M. M., Pienta, K. J., and Amend, S. R. (2020). Cancer cell foraging to explain bone-specific metastatic progression. *Bone* 2020:115788. doi: 10.1016/j.bone.2020.115788

Marusyk, A., and Polyak, K. (2010). Tumor heterogeneity: causes and consequences. *Biochim. Biophys. Acta.* 1805, 105–117. doi: 10.1016/j.bbcan.2009.11.002

Mboge, M. Y., Chen, Z., Khokhar, D., Wolff, A., Ai, L., Heldermon, C. D., et al. (2019). A non-catalytic function of carbonic anhydrase IX contributes to the glycolytic phenotype and pH regulation in human breast cancer cells. *Biochem. J.* 476, 1497–1513. doi: 10.1042/BCH20190177

McMeans, B. C., McCann, K. S., Guzzo, M. M., Bartley, T. J., Bieg, C., Blanchfield, P. J., et al. (2020). Winter in water: differential responses and the maintenance of biodiversity. *Ecol. Lett.* 23, 922–938. doi: 10.1111/ele.13504

McNickle, G. G., Wallace, C., and Baltzer, J. L. (2016). Why do mosses have height? Moss production as a tragedy of the commons game. *Evol. Ecol. Res.* 17, 75–93.

Michiels, C., Tellier, C., and Feron, O. (2016). Cycling hypoxia: a key feature of the tumor microenvironment. *Biochim. Biophys. Acta.* 1866, 78–86. doi: 10.1016/j.bbcan.2016.06.004

Miller, A. K., Brown, J. S., Basanta, D., and Huntly, N. (2020). What is the storage effect, why should it occur in cancers, and how can it inform therapy? *Cancer Control* 27:1073274820941968. doi: 10.1177/1073274820941068

Miller, A. K., Brown, J. S., Enderling, H., Basanta, D., and Whelan, C. J. (2021). The evolutionary ecology of dormancy in nature and in cancer. *Front. Ecol. Evol.* 9:676802. doi: 10.2289/fevo.2021.676802

Miller-Rushing, A. J., Primack, R. B., Templer, P. H., Rathbone, S., and Mukunda, S. (2009). Long-term relationships among atmospheric CO2, stomata, and intrinsic water use efficiency in individual trees. *Am. J. Bot.* 96, 1779–1786. doi: 10.3732/ajb.0800410

Morozov, A. Y. (2010). Emergence of Holling type III zooplankton functional response: bringing together field evidence and mathematical modelling. *J. Theor. Biol.* 265, 45–54. doi: 10.1016/j.jtbi.2010.04.016

Morris, D. W. (2003). Toward an ecological synthesis: a case for habitat selection. *Oecologia* 136, 1–13. doi: 10.1007/s00442-003-1241-4

Neftel, C., Laffy, J., Filbin, M. G., Hara, T., Shore, M. E., Rahme, G. J., et al. (2019). An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell* 178, 835–849.

Oke, T. A., and Turetsky, M. R. (2020). Evaluating *Sphagnum traits* in the context of resource economics and optimal partitioning theories. *Oikos* 129, 1204–1215. doi: 10.1111/oik.07195

Olsson, O., and Brown, J. S. (2010). Smart, smarter, smartest: foraging information states and coexistence. *Oikos* 119, 292–303. doi: 10.1111/j.1600-0706.2009.17784.x

Oyugi, J. O., Brown, J. S., and Whelan, C. J. (2012). Foraging behavior and coexistence of two sunbird species in a Kenyan woodland. *Biotropica* 44, 262–269. doi: 10.1111/j.1744-7429.2011.00785.x

Pachepsky, E., Nisbet, R. M., and Murdoch, W. W. (2008). Between discrete and continuous: consumer–resource dynamics with synchronized reproduction. *Ecology* 89, 280–288. doi: 10.1890/07-0641.1

Parker, T. M., Henriques, V., Beltran, A., Nakshatri, H., and Gogna, R. (2020). Cell competition and tumor heterogeneity. *Sem. Cancer Biol.* 63, 1–10. doi: 10.1016/j.semcan.2019.09.003

Paul, C. D., Mistriotis, P., and Konstantopoulos, K. (2017). Cancer cell motility: lessons from migration in confined spaces. *Nat. Rev. Cancer* 17, 131–140. doi: 10.1038/nrc.2016.123

Perfahl, H., Byrne, H. M., Chen, T., Estrella, V., Alarcon, T., Lapin, A., et al. (2013). "Multiscale modelling of angiogenesis and vascular tumour growth," in *Micro and Nano Flow Systems for Bioanalysis (Advanced Materials, Methods, and Devices)*, vol. 2, eds. M. Collins, and C. Koenig (New York, NY: Springer), 29–48. doi: 10.1007/978-1-4614-4376-6_3

Pressley, M., Gallaher, J. A., Brown, J. S., Tomaszewski, M. R., Borad, P., Damaghi, M., et al. (2021). Cycling hypoxia selects for constitutive HIF stabilization. *Sci. Rep.* 11:5777. doi: 10.1038/s414598-021-85184-8

Pulliam, H. R. (1974). On the theory of optimal diets. *Am Nat.* 108, 59–74. doi: 10.1086/282885

Rademakers, S. E., Lok, J., van der Kogel, A. J., Bussink, J., and Kaanders, J. H. A. M. (2011). Metabolic markers in relation to hypoxia; staining patterns and colocalization of pimonidazole, HIF-1α, CAIX, LDH-5, GLUT-1, MCT1 and MCT4. *BMC Cancer* 11:167. doi: 10.1186/1471-2407-11-167

Ravi, M., Paramesh, V., Kaviya, S. R., Anuradha, E., and Solomon, F. P. (2015). 3D cell culture systems: advantages and applications. *J. Cell. Phys.* 230, 16–26. doi: 10.1002/jcp.24683

Richards, S. A., Nisbet, R. M., Wilson, W. G., and Possingham, H. P. (2000). Grazers and diggers: exploitation competition and coexistence among foragers with different feeding strategies on a single resource. *Am. Nat.* 155, 266–279. doi: 10.1086/303316

Robertson-Tessi, M., Gillies, R. J., Gatenby, R. A., and Anderson, A. R. A. (2015). Impact of metabolic heterogeneity on tumor growth, invasion, and treatment outcomes. *Cancer Res.* 75, 1567–1579. doi: 10.1158/0008-5472.CAN-14-1428

Salcher, M. M. (2014). Same same but different: ecological niche partitioning of planktonic freshwater prokaryotes. *J. Limnol.* 73, 74–87.

Sasmita, A. O., Wong, Y. P., and Ling, A. P. K. (2018). Biomarkers and therapeutic advances in glioblastoma multiforme. *Asia Pac. J. Clin. Oncol.* 14, 40–51. doi: 10.1111/ajco.12756

Saxena, K., and Jolly, M. K. (2019). Acute vs. chronic vs. cyclic hypoxia: their differential dynamics, molecular mechanisms, and effects on tumor progression. *Biomolecules* 9:339. doi: 10.3390/biom9080339

Schaffer, W. M., Jensen, D. B., Hobbs, D. E., Gurevitch, J., Todd, J. R., and Schaffer, M. V. (1979). Competition, foraging energetics, and the cost of sociality in three species of bees. *Ecology* 60, 976–987. doi: 10.2307/1936866

Schoener, T. W. (1974). Resource partitioning in ecological communities. *Science* 5, 27–39. doi: 10.1126/science.185.4145.27

Schuster, B., Junkin, M., Kashaf, S. S., Romero-Calvo, I., Kirby, K., Matthews, J., et al. (2020). Automated microfluidic platform for dynamic and combinatorial drug screening of tumor organoids. *Nat. Commun.* 11:5271.

Schwinning, S., Litvak, M. E., Pockman, W. T., Pangle, R. E., Fox, A. M., Huang, C. W., et al. (2020). A 3-dimensional model of *Pinus edulis* and *Juniperus monosperma* root distributions in New Mexico: implications for soil water dynamics. *Plant Soil* 450, 337–355. doi: 10.1007/s11104-020-04446-y

Shembrey, C., Huntington, N. D., and Hollande, F. (2019). Impact of tumor and immunological heterogeneity on the anti-cancer immune response. *Cancers* 11:1217. doi: 10.3390/cancers11091217

Stewart, F. M., and Levin, B. R. (1973). Partitioning of resources and the outcome of interspecific competition: a model and some general considerations. *Am. Nat.* 954, 171–198. doi: 10.1086/282825

Valcourt, J. R., Lemons, J. M., Haley, E. M., Kojima, M., Demuren, O. O., and Coller, H. A. (2012). Staying alive: metabolic adaptations to quiescence. *Cell Cycle* 11, 1680–1696. doi: 10.4161/cc.19879

Venner, S., Pélisson, P. F., Bel-Venner, M. C., Débias, F., Rajon, E., and Menu, F. (2011). Coexistence of insect species competing for a pulsed resource: toward a unified theory of biodiversity in fluctuating environments. *PLoS One* 6:e18039. doi: 10.1371/journal.pone.0018039

Verhaak, R. G. W., Hoadley, K. A., and Purdom, E. (2010). Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 17, 98–110. doi: 10.1016/j.ccr.2009.12.020

Vincent, T. L. S., Scheel, D., Brown, J. S., and Vincent, T. L. (1996). Trade-offs and coexistence in consumer-resource models: it all depends on what and where you eat. *Am. Nat.* 148, 1038–1058. doi: 10.1086/285970

Werner, E. E., and Hall, D. J. (1977). Competition and habitat shift in two sunfishes (Centrarchidae). *Ecology* 58, 869–876. doi: 10.2307/1936222

West, A. G., Hultine, K. R., Jackson, T. L., and Ehleringer, J. R. (2007). Differential summer water use by *Pinus edulis* and *Juniperus osteosperma* reflects contrasting hydraulic characteristics. *Tree Phys.* 27, 1711–1720. doi: 10.1093/treephys/27.12.1711

White, E. Z., Pennant, N. M., Carter, J. R., Hawsawi, O., Odero-Marah, V., and Hinton, C. V. (2020). Serum deprivation initiates adaptation and survival to oxidative stress in prostate cancer cells. *Sci. Rep.* 10:12505. doi: 10.1038/s41598-020-68668-x

Wilson, W. G., Osenberg, C. W., Schmitt, R. J., and Nisbet, R. M. (1999). Complementary foraging behaviors allow coexistence of two consumers. *Ecology* 80, 2358–2372. doi: 10.1890/0012-9658(1999)080[2358:cfbaco]2.0.co;2

Wolfe, J. O. (1996). Coexistence of white-footed mice and deer mice may be mediated by fluctuating environmental conditions. *Oecologia* 108, 529–533. doi: 10.1007/bf00333730

Wooten, D. J., Groves, S. M., Tyson, D. R., Liu, Q., Lim, J. S., Albert, R., et al. (2019). Systems-level network modeling of small cell lung cancer subtypes identifies master regulators and destabilizers. *PLoS Comp. Biol* 15:e1007343. doi: 10.1371/journal.pcbi.1007343

Xiao, X., and Fussmann, G. F. (2013). Armstrong-McGehee mechanism revisited: competitive exclusion and coexistence of nonlinear consumers. *J. Theor. Biol.* 339, 26–35. doi: 10.1016/j.jtbi.2013.05.025

Yang, Y., Sun, M., Wang, L., and Jiao, B. (2013). HIFs, angiogenesis, and cancer. *J. Cell. Biochem.* 114, 967–974. doi: 10.1002/jcb.24438

Yeo, S. K., and Guan, J. L. (2017). Breast cancer: multiple subtypes within a tumor? *Trends Cancer* 3, 753–760. doi: 10.1016/j.trecan.2017.09.001

Younes, M., Brown, R. W., Stephenson, M., Gondo, M., and Cagle, P. T. (1997). Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. *Cancer* 80, 1046–1051. doi: 10.1002/(sici)1097-0142(19970915)80:6<1046::aid-cncr6>3.0.co;2-7

Yu, M., Yongzhi, H., Chen, S., Luo, X., Lin, Y., Zhou, Y., et al. (2017). The prognostic value of GLUT1 in cancers: a systematic review and meta-analysis. *Oncotarget* 8, 43356–43367. doi: 10.18632/oncotarget.17445

Zepeda, V., and Martorell, C. (2019). Fluctuation-independent niche differentiation and relative non-linearity drive coexistence in a species-rich grassland. *Ecology* 100:e02726. doi: 10.1002/ecy.2726

**Conflict of Interest:** ML was employed by the company Inspirata, Inc.

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frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 08 October 2021
doi: 10.3389/fevo.2021.691830

# Cancer as a Model System for Testing Metabolic Scaling Theory

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Biological allometries, such as the scaling of metabolism to mass, are hypothesized to result from natural selection to maximize how vascular networks fill space yet minimize internal transport distances and resistance to blood flow. Metabolic scaling theory argues two guiding principles—conservation of fluid flow and space-filling fractal distributions—describe a diversity of biological networks and predict how the geometry of these networks influences organismal metabolism. Yet, mostly absent from past efforts are studies that directly, and independently, measure metabolic rate from respiration and vascular architecture for the same organ, organism, or tissue. Lack of these measures may lead to inconsistent results and conclusions about metabolism, growth, and allometric scaling. We present simultaneous and consistent measurements of metabolic scaling exponents from clinical images of lung cancer, serving as a first-of-its-kind test of metabolic scaling theory, and identifying potential quantitative imaging biomarkers indicative of tumor growth. We analyze data for 535 clinical PET-CT scans of patients with non-small cell lung carcinoma to establish the presence of metabolic scaling between tumor metabolism and tumor volume. Furthermore, we use computer vision and mathematical modeling to examine predictions of metabolic scaling based on the branching geometry of the tumor-supplying blood vessel networks in a subset of 56 patients diagnosed with stage II-IV lung cancer. Examination of the scaling of maximum standard uptake value with metabolic tumor volume, and metabolic tumor volume with gross tumor volume, yield metabolic scaling exponents of 0.64 (0.20) and 0.70 (0.17), respectively. We compare these to the value of 0.85 (0.06) derived from the geometric scaling of the tumor-supplying vasculature. These results: (1) inform energetic models of growth and development for tumor forecasting; (2) identify imaging biomarkers in vascular geometry related to blood volume and flow; and (3) highlight unique opportunities to develop and test the metabolic scaling theory of ecology in tumors transitioning from avascular to vascular geometries.

**Keywords: metabolic scaling, Kleiber's law, PET-CT image analysis, quantitative imaging biomarkers, lung cancer**

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***Edited by:***
*Christopher J. Whelan, Moffitt Cancer Center, United States*

***Reviewed by:***
*Caterina Guiot, University of Turin, Italy*
*Kenneth Gage, H. Lee Moffitt Cancer Center & Research Institute, United States*

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***Specialty section:***
*This article was submitted to Models in Ecology and Evolution, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *07 April 2021*
***Accepted:*** *13 September 2021*
***Published:*** *08 October 2021*

***Citation:***
*Brummer AB and Savage VM (2021) Cancer as a Model System for Testing Metabolic Scaling Theory. Front. Ecol. Evol. 9:691830. doi: 10.3389/fevo.2021.691830*

# 1. INTRODUCTION

Since Max Kleiber's finding of the remarkable biological pattern that organismal basal metabolic rate, $B$, scales with body mass, $M$, as $B \propto M^{3/4}$, scientists have worked to both understand and extend the phenomenon of metabolic scaling (Kleiber, 1932). Applications of metabolic scaling have permeated the biological sciences, spanning evolutionary and cellular biology (DeLong et al., 2010), predator-prey interactions at both the individual level (Pawar et al., 2012; Hatton et al., 2015) and at the trophic level (Brose et al., 2006), fish reproduction energetics (Barneche et al., 2018), forest structure, demography, and dynamics (Enquist et al., 2009; West et al., 2009), and species distribution modeling (Harte and Newman, 2014). Explanations for the origins of metabolic scaling in individual vascular organisms are numerous, and all center around functional optimization of a hierarchically branching vascular network that distributes and delivers resources throughout the body (West et al., 1997; Turcotte et al., 1998; Bejan, 2001; Banavar et al., 2010). The phenomenon of metabolic scaling has been studied through measurements of metabolism at either the whole organism level (Schmidt-Nielsen, 1984; Mori et al., 2010), or with predictions of metabolism rooted in vascular theory (Bentley et al., 2013; Lau et al., 2019; Brummer et al., 2021).

Surprisingly, we could not find a single study that examines metabolism from both of these perspectives for the same tissue, organ, or organism (Price et al., 2012). Furthermore, proposed theories that purport to explain the origins of metabolic scaling in vascular organisms fail to explain why the pattern persists in avascular organisms. To address these issues, we present simultaneous measurements of metabolic scaling in tumors derived from uptake of metabolic radio-tracers and of the vasculature that comprises and surrounds the tumors. Recent efforts to improve and expedite cancer diagnosis, treatment planning, and tracking responses have produced medical imaging and computer vision technologies that offer a unique lens with which to study metabolic scaling, particularly within tissues that have undergone the avascular-to-vascular transition. We show that insight from metabolic scaling theory can be leveraged to derive vascular-based biomarkers of cancer, potentially introducing an ensemble of biomarkers indicative of tumor growth and the distribution and flow of blood.

Radiological images of non-small cell lung cancer (NSCLC) are predominately analyzed from medical imaging as solid volumes of tissue absent of surrounding vessels (Gevaert et al., 2012; Aerts et al., 2014; Zhou et al., 2018; Ardila et al., 2019). Yet, when viewed at an enhanced scale, these volumes are seen to be entirely embedded in, and sometimes partially composed of, networks of vascular tissue that are distinguishable from surrounding healthy tissues (**Figure 1**) (Jain, 2005; Rao et al., 2016; Wang et al., 2017; Alilou et al., 2018). The radiomics paradigm of personalized medicine uses artificial intelligence and machine learning algorithms to detect and classify NSCLCs, and to track individual response to intervention and treatment. This approach requires large and accurate datasets of all possible biological features, or biomarkers, associated with disease (Lambin et al., 2017). The current practice within radiomics restricts the space of possible features to statistical measures regarding tumor volume, shape, and intensity variation—the latter being directly indicative of metabolism. This approach necessitates the existence of solid masses in order to facilitate detection, thus setting a fundamental limit on early detection (Pashayan and Pharoah, 2020). As a way to support existing metrics and to provide a more comprehensive view of the tumor environment, we propose a way forward that leverages the connectivity of the vessels that compose and surround an NSCLC and that incorporates results and insights from theory on tumor metabolism and growth. (West et al., 2001; Guiot et al., 2003, 2006; Herman et al., 2011; Milotti et al., 2013; Ribeiro et al., 2017; Pérez-García et al., 2020).

Established theory predicts average empirical branching properties at the whole-network level by minimizing energy to pump and distribute blood and ensuring that vessels efficiently reach and feed all cells. However, there exists wide variation around predictions for these average properties. Variation in measures of vessel branching, connectivity, and scaling have been shown to serve as biomarkers of disease (Yao et al., 2011; Huang et al., 2018; Pandey et al., 2018; Apte et al., 2019). Furthermore, there exist competing theories relating organ and organismal growth and metabolism to vascular branching patterns—relationships that result from the optimization of fluid transport and resource distribution (West et al., 1997; Zamir, 2006; Savage et al., 2008; Huo and Kassab, 2009a,b; Banavar et al., 2010; Dodds, 2010). Extensions of these theories predict the growth trajectories of tumors, incorporating the angiogenic transition from avascular, diffusion-dominated growth to vascularized growth that often precedes metastasis (West et al., 2001; Guiot et al., 2003, 2006; Herman et al., 2011; Milotti et al., 2013; Ribeiro et al., 2017; Pérez-García et al., 2020). Yet, no rigorous application of these theories has been conducted to examine energetic measures of metabolic scaling in tumors to the underlying vasculature supplying tumor growth and maintenance. We analyze clinical human NSCLC X-ray images to identify the vascular branching features most prevalent with NSCLC presence. We then map these vascular patterns to variation in the metabolic scaling of tumors as measured from metabolic imaging techniques of nuclear medicine.

# 2. MATERIALS AND METHODS

## 2.1. PET-CT Imaging

As tumors undergo rapid cellular proliferation they subsequently have higher metabolic demands. The deregulated uptake of glucose to sustain growth and maintenance in tumors can be observed with the nuclear imaging technique of positron emission tomography (PET). The radioactive tracer [$^{18}$F]fluro-2-deoxyglucose ($^{18}$F-FDG) is utilized to measure glucose uptake in tumors in clinical settings. The standard uptake values (SUV) of glucose uptake are measured as $SUV = r/(a'/w)$, where $r$ is the concentration of radioactivity (measured in kiloBecquerels per milliliter, kBq/mL) detected, $a'$ is the radioactivity of the full volume of injected radio-tracer (kBq) adjusted for radioactive decay since injection, and $w$ is the weight of the patient (g).

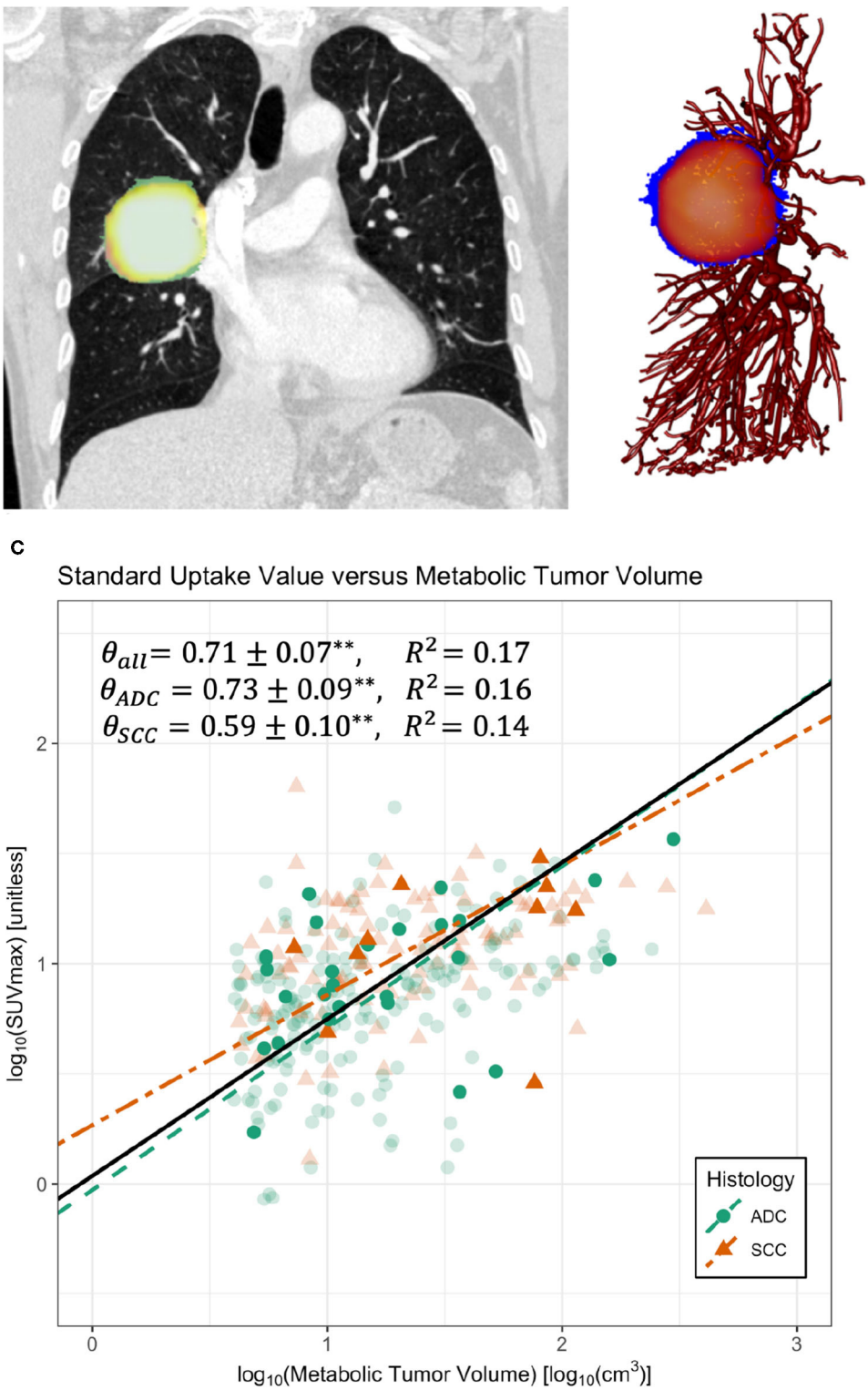


**FIGURE 1 |** Co-registered PET-CT images **(A)** and sample skeletonization of pulmonary vasculature with tumor **(B)**. **(C)** Regression on maximum standard uptake value ($SUV_{max}$) and metabolic tumor volume (*MTV*) for all data compiled (black solid line), divided into the histological categories of adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Regressions demonstrate a significant scaling relationship between $SUV_{max}$ and *MTV* with scaling exponents approximately equal to 0.71 (**Table 1**). **Indicates $p < 0.005$. Bold symbols represent data with accompanying clinical imaging used in vascular analyses, whereas transparent symbols represent data collected without original clinical imaging. Data was truncated at $MTV \approx 4cm^3$ to avoid errors from the partial volume effect.

Importantly, implicit assumptions are made that tissue imaged has a density of 1 g/mL, and that, aside from the more highly metabolic tissues of the brain, liver, and tumors, the radio-tracer injected is uniformly distributed throughout the body. Thus, an SUV of 1 can be loosely interpreted as normal (Kinahan and Fletcher, 2010). Due to the potential for variation between individuals, imaging machines and imaging protocols, SUV measures are presently used as a qualitative biomarker for disease to examine relative metabolic demand.

Advances in medical imaging technology allow for the simultaneous use of PET and X-ray computerized tomographic (PET-CT) imaging to extract overlapping images of tissue metabolism and soft-to-hard tissue presence. When coupled with computer vision software, collections of two-dimensional image "slices" can be reconstructed into three-dimensional volumes from which spatial patterns can be extracted for disease diagnostics. These technologies are not without crucial limitations. The two-dimensional image slices have a finite resolution and thickness, and consequently errors can occur when examining structures near these resolution-thickness limits. These errors are broadly categorized as partial-volume effects (PVE), and require careful consideration (Soret et al., 2007).

In order to introduce typical imaging metrics, it is important to first consider any volume or region of interest (ROI) as being subdivided into a collection of $N$ cubes, or voxels, of uniform volume. These voxel volumes are determined by the image slice thickness and resolution. Importantly, CT imaging maps the spatial distribution of biologic structures, while PET imaging maps the metabolic uptake of those structures. Any given ROI will have a total volume $V$ as determined from CT imaging as the sum of each voxel volume $v_i$ within that large volume. Each voxel volume has its own corresponding $SUV$ measure, $SUV_i$, determined from PET imaging. Conventional tumor evaluation involves measuring the following: total, or gross tumor volume ($GTV$) as the sum of all voxel volumes $v_i$ that comprise the tumor *as observed strictly from CT imaging*. Metabolic tumor volume ($MTV$) is the sum of all voxel volumes $v_i$ that comprise the tumor with a corresponding $SUV_i$ exceeding a conventional threshold of 2.5. Total lesion activity (TLA) is the summed product of voxel volume and $SUV_i$, or $TLA = \sum_i SUV_i v_i$. Tumor size is a common clinical metric determined from semi-major and semi-minor tumor diameters. Finally, various summary statistics for $SUV$ may be computed over a whole ROI, such as maximum, $SUV_{max}$, median, $SUV_{med}$, mean, $SUV_{mean}$, or over a temporal range, such as peak $SUV_{peak}$ (Bailey et al., 2005; Valk et al., 2006). In radiomics studies, this small collection of metrics quickly runs into the hundreds, as many metrics are analyzed as spatial distributions with many accompanying statistical features.

## 2.2. Establishment of Metabolic Scaling

In this work we compiled data from four separate studies of PET-CT imaging of NSCLC patients (Furumoto et al., 2018; Mattonen et al., 2019; Chardin et al., 2020; Pérez-García et al., 2020). These studies consisted of pre-treatment PET-CT scans for 535 patients, of which 401 were adenocarcinoma and 134 were squamous cell carcinoma. Imaging acquisition and patient information for each study can be found in the original publications. The metrics we chose to focus on were $SUV_{max}$ as a measure of metabolism, $MTV$ as a measure of glucose consuming tumor volume, and $GTV$ as a measure of total tumor volume that includes glucose consuming tissue in addition to all other tumor tissues (e.g., metabolically active but glucose inactive tissues, and necrotic tissues). $SUV_{max}$ was chosen over other $SUV$ features as it is less susceptible to variation in delineation of the tumor ROI.

Measures of maximum standard uptake value ($SUV_{max}$) and metabolic tumor volume ($MTV$) were graphed on a log-log scale to identify the existence of a scaling relationship between these variables (**Figure 1**). In addition to regressing on the whole dataset, these data were also categorized by the histological classifications of adenocarcinoma (ADC) and squamous cell carcinoma (SCC) to allow for possible variations in metabolic scaling due to tumor heterogeneity. Standard major axis regressions were performed as interest is primarily on the regression slope, and the axes of variation have fundamentally different units. Of the data collected, a subset have the original PET-CT imaging available on The Cancer Imaging Archive (Clark et al., 2013; Prior et al., 2013). For this subset of data, log-log graphs were analyzed to investigate scaling between $SUV_{max}$ and $MTV$ as well as $MTV$ and gross tumor volume ($GTV$) (**Figure 2**). Regression statistics are located in **Table 1**. To account for the partial volume effect associated with small voxel thresholding in PET imaging, we analyzed the data by imposing a hard threshold on tumors less than $4cm^3$ in volume. Tumors smaller than this volume are known to exhibit greater than 10% error on measurements of $SUV_{max}$ from PET imaging due to the partial volume effect (Soret et al., 2007; Kinahan et al., 2009). After filtering for the partial volume effect, left for analysis were 207 ADC and 109 SCC data points.

## 2.3. Segmentation of CT Images for Vascular Measurement

This data is part of the Radiogenomics dataset from Gevaert et al. (2012) and Zhou et al. (2018) and consists of CT scans of 211 NSCLC patients with manual annotations delineating tumor boundaries and PET-CT imaging available for 150 of these patients. We selected patients with clinical staging of II or greater to ensure tumors were sufficiently large enough for identifiable vasculature. Within this group we examined vasculature where pulmonary vessels could be easily identified as supplying tumors with blood, resulting in 56 patients.

Image processing prior to segmentation is crucial for expediency and accuracy. To extract only lung interior regions of interest, we implemented the watershed technique (Shojaii et al., 2005; D'Sa et al., 2019) with a black top-hat transform for re-inclusion of juxtapleural nodes and near-hilar vessels (Singadkar et al., 2018). This routine is followed by contrast limited adaptive histogram equalization (CLAHE) (Jin et al., 2001) and iterated global thresholding (Samet and Yildirim, 2016) to enhance the signal to noise ratio (**Figure 3**).

Segmentation of vasculature is accomplished using the open-source software Angicart, developed by the Savage Lab at UCLA (Newberry et al., 2015; Brummer et al., 2021). Angicart software

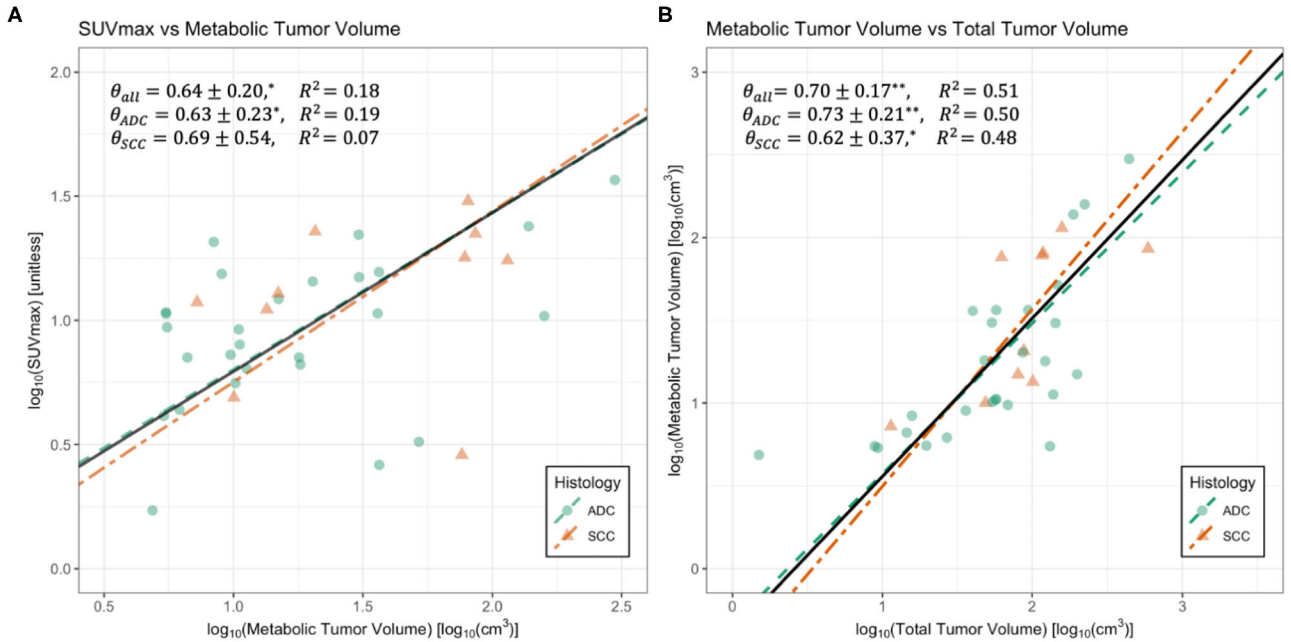


**FIGURE 2 |** Regressions for maximum standard uptake value ($SUV_{max}$) vs. metabolic tumor volume (*MTV*) **(A)** and MTV vs. gross tumor volume (*GTV*) **(B)**. Data is divided by the histological classifications of adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Black solid lines represent regressions on whole datasets. Summaries of regressions are presented in **Table 1**. *Indicates $p < 0.05$, and ** indicates $p < 0.005$. Standard major axis regression shows that both methods result in significant and consistent measurements of metabolic scaling exponents.

**TABLE 1 |** Estimates of metabolic scaling exponents.

| | Metabolic scaling exponent measurements ($\theta$) | | | |
|---|---|---|---|---|
| **Histology** | $SUV/MTV_{meta}$ | $SUV/MTV_{subset}$ | $MTV/GTV_{subset}$ | **Vascular est.** |
| Adenocarcinoma | 0.73 ± 0.09** | 0.63 ± 0.23* | 0.73 ± 0.21** | 0.85 ± 0.03 |
| Squamous cell carcinoma | 0.59 ± 0.10** | 0.69 ± 0.54 | 0.62 ± 0.37* | 0.87 ± 0.02 |
| Combined | 0.71 ± 0.07** | 0.64 ± 0.20* | 0.70 ± 0.17** | 0.85 ± 0.06 |

*Significance of regression based exponents are indicated with asterisks, where * indicates $p < 0.05$, and ** indicates $p < 0.005$. Sample sizes are: meta-analysis dataset $N_{ADC} = 207, N_{SCC} = 109$; subset analysis $N_{ADC} = 27, N_{SCC} = 10$; vascular-based estimates $N_{ADC} = 54, N_{SCC} = 20$. Note that the vascular estimates do not have p-values as they were calculated directly from vascular data as opposed to regression data. Also, all regressions performed were standard major axis regressions to accommodate differences in axes' units as well as the emphasis being on the value of the regression slope (Newberry et al., 2015).*

reconstructs digital representations of vascular networks from medical images of any modality. The segmentation routine uses a spherical-growth algorithm to map the vascular network. It is a fully automated software (as defined in Myatt et al., 2012) that only requires vessels of interest to be brighter on a grayscale than surrounding tissues. Angicart output consists of vessel radii, lengths, branching angles, connectivity, and centerline coordinates. Angicart results have been published for: $\mu$CT mouse lung data, human thoracic CT scans, and pulmonary vasculature (Newberry et al., 2015; Tekin et al., 2016; Brummer et al., 2021).

### 2.3.1. Errors From the Segmentation and Skeletonization Procedures

Three types of errors in the data acquisition process consisted of: (1) individual vessels disconnected from vascular trees, (2) nonvascular tissue misidentified as vascular trees (3) misidentification of vascular tree roots.

The segmentation procedure can produce disconnected individual artifacts that Angicart automatically identifies as singular vessels. As the framework of metabolic scaling theory relies on vascular networks, such artifacts are simply filtered from the resulting skeletonization. Similarly, certain non-vascular tissues that pass the segmentation procedure may result in erroneous vascular trees. Common examples of such errors are non-vascular tissues in the hilar and sternal regions of the lungs. These errors are easily identified and removed manually.

Finally, misidentification of vascular tree roots occurs due to programming in Angicart intended to identify roots based on vessel radius, an assumption based on models of healthy vascular networks. This assumption does not hold in this study however as pulmonary vascular networks embedded with

tumors are known to have vessels with unusually large diameters within the boundary of the tumor (Wang et al., 2017). Thus, to identify vascular tree roots, we calculated the geometric centroid of all vessel coordinates for each half of the lungs in an effort to approximate the location of the hilar vessel roots. Vascular tree roots were then identified as those nearest to the centroid position.

## 2.4. Modeling Vascular Data

Metabolic scaling theory is a first principles model linking biologic scaling phenomena to hierarchically branching resource distribution networks. It was initially proposed as a model for Kleiber's law—the scaling of organismal metabolism, $B$, with body mass, $m$, expressed as $B = B_0 m^{\theta}$ (Kleiber, 1932; West et al., 1997). It has since been applied to a myriad of systems spanning plant metabolism, forest demography, city scaling, and organismal growth and development (Enquist et al., 1998; West et al., 1999, 2001; Bettencourt et al., 2007). Importantly, both theoretical and empirical studies have demonstrated allometric relationships based on vascular branching within many different organs and tissue types, spanning heart, lungs, cerebral arterial trees, muscle tissue, and the torso (Majumdar et al., 2005; Huo and Kassab, 2009a; Wright et al., 2013). Despite apparent differences in absolute metrics such as blood pressure, flow resistance, and vessel number-density, a crucial component of vascular branching from the perspective of biological allometries is that they provide scale-free metrics of different organs and tissues. Thus, the combination of scale-dependent and organ-specific metrics (e.g., diameter, length, and pressure at the initial and terminal generations) and scale-free metrics (e.g., ratios of vessel diameters and lengths) can provide functional information related to biologic rates, namely metabolism. Here we summarize the pertinent variables used to describe vascular branching in metabolic scaling theory, the mechanistic constraints that predict values for these variables, and how metabolic scaling theory can be applied to investigate PET-CT imaging data for NSCLC. For further background, see (West et al., 2001; Savage et al., 2008; Herman et al., 2011; Brummer et al., 2017, 2021).

### 2.4.1. Branching Variables

Metabolic scaling theory idealizes vascular trees as having cylindrically shaped, pipe-like branching architectures (**Figure 4A**). Here, the fundamental units are individual bifurcations consisting of a parent vessel that divides into two child vessels. Any vessel can be parameterized by its radius, $r$, length, $l$, and branching generation, $j$, the latter of which takes the value $j = 0$ at the root and $j = N$ for an $N$ generational network. We next define the following asymmetric scale factors: the *average* and *difference radial scale factors* $\bar{\beta} = (r_{j,\mu} + r_{j,\nu})/(2r_{j-1})$ and $\Delta\beta = (l_{j,\mu} - l_{j,\nu})/(2l_{j-1})$, and the *average* and *difference length scale factors* $\bar{\gamma} = (l_{j,\mu} + l_{j,\nu})/(2l_{j-1})$ and $\Delta\gamma = |l_{j,\mu} - l_{j,\nu}|/(2l_{j-1})$.

These four scale factors can be constrained through two optimizations that: (1) maximize the number of capillaries per unit volume of tissue and (2) minimize the resistance to fluid flow. Here we outline the conceptual arguments for these constraints at the single bifurcation level. Maximizing capillaries per unit volume is done by modeling the system as a space-filling fractal (Mandelbrot, 1982; Barnsley, 2012). In the context of vascular branching systems, this can be demonstrated through an iterative process (Savage et al., 2008). To supply blood to the $N_N$ terminal vessels in generation $N$, each comprising a blood volume of $v_N$, the $N_{N-1}$ vessels of the preceding generation $N-1$ must have a matching volume of blood across all vessels, each with a blood volume of $v_{N-1}$. Iterating this argument across multiple generations results in the expression that $N_N v_N = N_{N-1} v_{N-1} = \ldots = N_0 v_0$. To apply this argument at the level of a single bifurcation, we approximate the blood service volumes by the vessel lengths, $v_N \propto l_N^3$, allow for asymmetric branching, and express this iterative argument instead by considering first a generic parent service volume in generation $j-1$ that supplies all child vessels distal to it, yielding $l_{j-1} = (l_{j,\mu}^3 + l_{j,\nu}^3)^{1/3}$. Writing this expression in terms of the asymmetric branching scale factors yields,

$$1 = (\bar{\gamma} + \Delta\gamma)^3 + (\bar{\gamma} - \Delta\gamma)^3 \tag{1}$$

Minimizing the resistance to fluid flow results in two separate constraints depending on whether the flow is pulsatile—with resistance $Z_j \propto 1/r_j^2$—or laminar—with resistance $Z_j \propto l/r_j^4$. In pulsatile flow, reflections can occur as pulses cross a bifurcation. Thus, impedance matching across a given bifurcation minimizes reflections and results in,

$$1 = (\bar{\beta} + \Delta\beta)^2 + (\bar{\beta} - \Delta\beta)^2 \tag{2}$$

Of note, Equation (2) preserves the cross-sectional area from a parent vessel to its child vessels, which results in a constant blood flow rate across the bifurcation. For laminar flow, resistance due to friction is minimized, which results in,

$$1 = (\bar{\beta} + \Delta\beta)^3 + (\bar{\beta} - \Delta\beta)^3 \tag{3}$$

In Equation (3) the cross-sectional area increases from a parent vessel to its child vessels, which subsequently slows the rate of blood flow across the bifurcation. We note that Equation (3) is a variation on the canonical Murray's Law (Murray, 1926), only here the vessels radii have been expressed in terms of the radial branching scale factors $\bar{\beta}$ and $\Delta\beta$.

We also examined the Horton-Strahler length scale factor, $\gamma_{HS}$, a measure of length scaling that originates from an alternative generational labeling scheme first proposed in (Horton, 1945; Strahler, 1957) and examined in greater detail in (Yekutieli and Mandelbrot, 1994; Turcotte et al., 1998; Eloy et al., 2017). This scheme starts with labeling all identified terminal tips as the starting generation $N = 1$, and, working upstream toward the root vessel, advances the generation index only when two equally labeled vessels merge, as shown in **Figure 4B**. After labeling, vessels are redefined by their Horton-Strahler index such that a "new" vessel does not "begin" unless the Horton-Strahler index has changed. This relabeling between *canonical generation* (CG) labeling and *Horton-Strahler* (HS) labeling is demonstrated in **Figures 4C,D**. In **Figure 4E**, we compare distributions of the symmetrically defined length scale factor from CG labeling $\gamma_{CG} =$

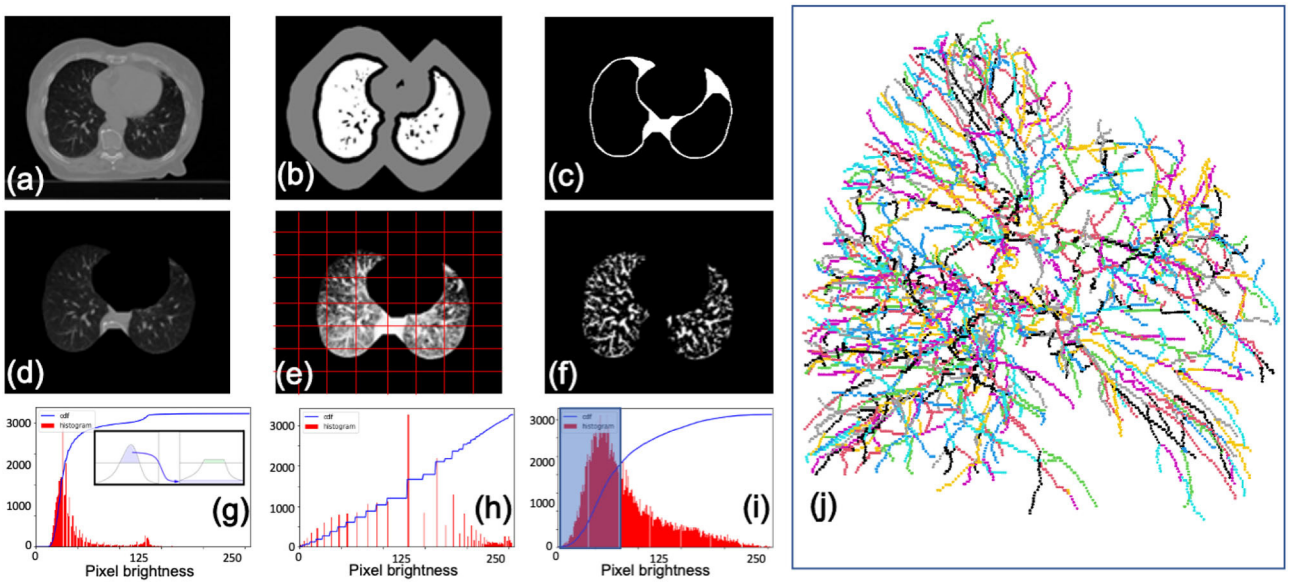


**FIGURE 3 |** Segmentation procedure for CT scans. **(a)** Initial image. **(b)** Lung boundary markers determined using watershed. **(c)** Mask with boundaries re-included by black top-hat transform. **(d)** Segmented image. **(e)** Contrast enhanced image with adaptive mesh (red) overlaid for limited equalization (CLAHE). **(f)** Final image after enhancement and de-noising via thresholding. Examples of pixel brightness histograms prior to enhancement **(g)**, after enhancement **(h)**, and with a de-noising filter **(i)**. **(j)** Left lung vessel skeletonization output from Angicart following steps described previously. Vessels are colored by canonical generation labeling (see **Figure 4**).

**TABLE 2 |** Summaries of vessel network properties.

| Metric | Tissue supplied by vessel tree | | | Sym. MST pred. |
|---|---|---|---|---|
| | ADC | SCC | Healthy tissue | |
| Number of generations | 3.58 ± 0.08 | 4.02 ± 0.07 | 5.23 ± 0.16 | $N$ |
| Number of terminal tips | 13.84 ± 0.99 | 14.62 ± 0.44 | 49.11 ± 4.50 | $2^N$ |
| Root length (*mm*) | 18.72 ± 1.26 | 18.41 ± 0.90 | 17.59 ± 1.17 | $l_0$ |
| Root radius (*mm*) | 1.23 ± 0.03 | 1.15 ± 0.02 | 1.12 ± 0.03 | $r_0$ |
| Root volume ($mm^3$) | 91.84 ± 6.52 | 70.31 ± 3.33 | 71.86 ± 5.28 | $r_0^2 l_0$ |
| Tip length (*mm*) | 10.26 ± 0.89 | 9.55 ± 0.77 | 11.28 ± 0.87 | $l_N = \bar{\gamma}^N l_0$ |
| Tip radius (*mm*) | 0.98 ± 0.02 | 0.97 ± 0.02 | 0.78 ± 0.01 | $r_N = \bar{\beta}^N r_0$ |
| Tip volume ($mm^3$) | 31.40 ± 2.79 | 29.13 ± 2.50 | 22.35 ± 2.11 | $r_N^2 l_N$ |
| Average radial scale factor | 0.85 ± 0.02 | 0.85 ± 0.02 | 0.75 ± 0.01 | $\bar{\beta} \approx 0.71$ |
| Difference radial scale factor | -0.06 ± 0.01 | -0.06 ± 0.01 | -0.06 ± 0.01 | $\Delta\beta = 0$ |
| Average length scale factor | 1.59 ± 0.22 | 1.72 ± 0.25 | 1.73 ± 0.24 | $\bar{\gamma} \approx 0.79$ |
| Difference length scale factor | 0.45 ± 0.06 | 0.49 ± 0.06 | 0.44 ± 0.06 | $\Delta\gamma = 0$ |
| Horton-Strahler length scale factor | 0.65 ± 0.11 | 0.73 ± 0.32 | 0.48 ± 0.07 | $\gamma_{HS} \approx 0.79$ |
| Volumetric scale factor | 2.41 ± 0.36 | 2.52 ± 0.34 | 2.05 ± 0.27 | $\nu \approx 0.79$ |

*Measurements are for the vascular networks supplying either adenocarcinomas (ADC), squamous cell carcinomas (SCC), or healthy tissue. These measurements are compared to predictions from the symmetric metabolic scaling theory, in which all sibling vessels are identical ($\Delta\beta = \Delta\gamma = 0$). Reported values are geometric means with associated standard errors.*

$l_j/l_{j-1}$, which ignores sibling branch variation, to the length scale factor from HS labeling $\gamma_{HS} = l_{HS,j}/l_{HS,j-1}$.

Measurements of the branching scale factors, $\bar{\beta}, \bar{\gamma}, \Delta\beta$, and $\Delta\gamma$, were made for all segmented pulmonary vessels. Additionally, the following branching network metrics were collected: number of generations and number of terminal vessels across all identified vascular networks; root-vessel length, radius, and volume; terminal vessel length, radius, and volume. Two additional metrics that were collected were the Horton-Strahler length scale factor and the volumetric scale factor. Summary statistics for vascular network metrics collected are presented in **Table 2**. The volumetric scale factor represents the scaling of blood volume across a bifurcation, and is defined as $\nu = 2\bar{\beta}^2\bar{\gamma} + 4\bar{\beta}\Delta\beta\Delta\gamma + 2\bar{\gamma}\Delta\beta^2$. This metric is informative for examining how vascular based estimates of metabolic scaling vary with network size.

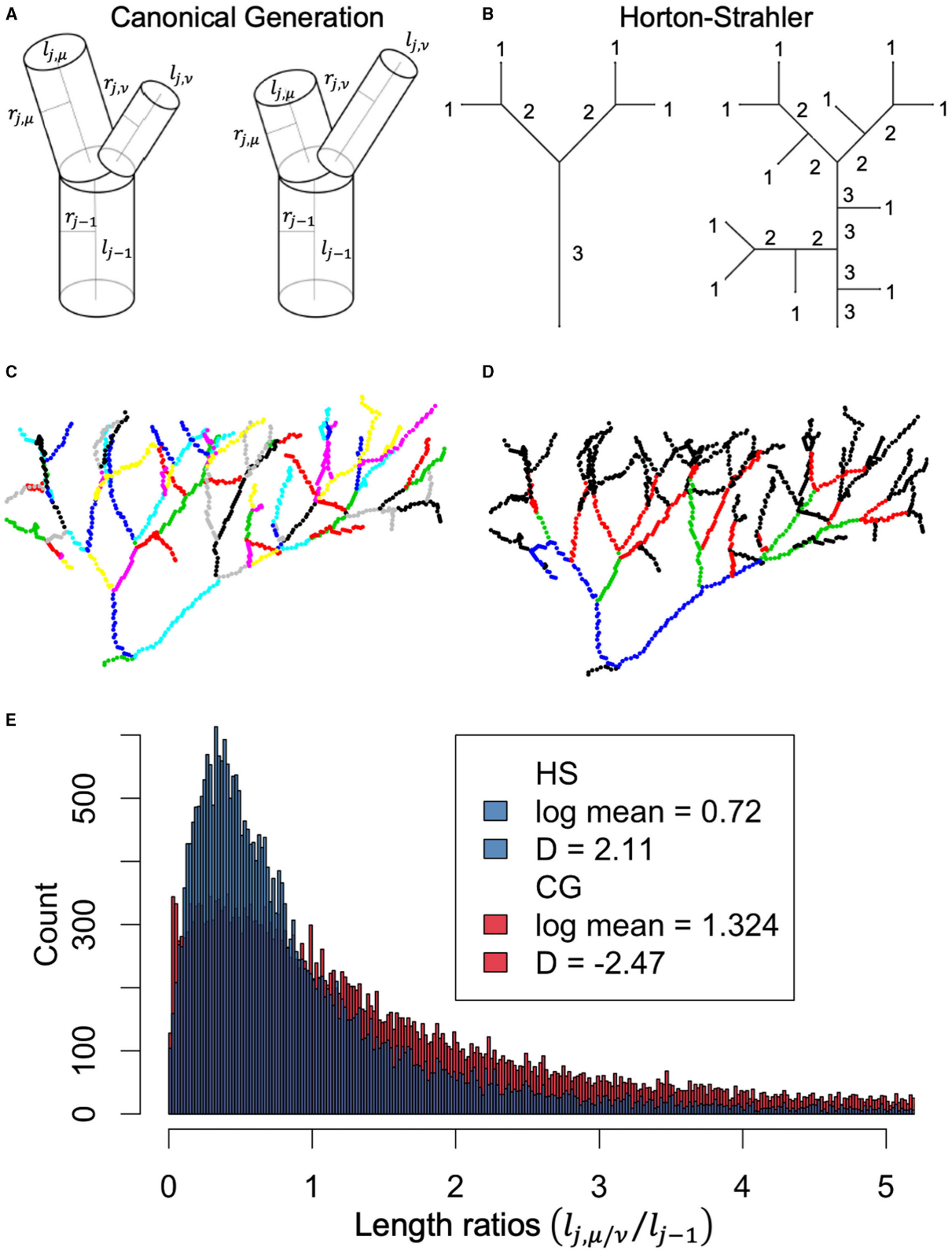


**FIGURE 4 | (A)** Canonical generation (CG) labeling for asymmetric bifurcation demonstrates how vessel endpoints are determined by presence of bifurcation. **(B)** Horton-Strahler (HS) labeling demonstrating how vessel endpoints are determined by changes in HS label. **(C)** Example vessel network color coded by different

*(Continued)*

**FIGURE 4 |** vessels labeled according CG method. **(D)** Same network as in **(C)**, now color coded by difference vessels defined according to HS labeling. **(E)** Histogram of length-scale factors calculated using CG labeling (red) or HS labeling (blue). Importantly, metabolic scaling theory predicts that vessel lengths branch according to $l_{j-1}^{D} = l_{j,\mu}^{D} + l_{j,\nu}^{D}$, where $D$ represents the fractal dimension and should be equivalent to the Euclidean dimension of the space being filled. Estimates of the fractal dimension using HS labeling fall within the expected range of $D \in [2, 3]$, compared to the non-intuitive value of $D = -2.47$ for CG labeling.

### 2.4.2. Measurement Procedures

To examine patterns and variations in vascular branching features between vessels that supply tumor tissue and those that supply healthy tissue, vessels first had to be categorized into these two different groups. To identify vessels directly responsible for supplying tumors, we identified all vessels from the Angicart segmentation output that intersected with the manual annotation of the tumor boundary. Next, all parent vessels to those intersecting the tumor boundary were added to the group of tumor supplying vessels. This process was iterated until reaching the vessel root for any given pulmonary vessel network. For multiple examples of tumor supplying vessels identified in this manner, see **Figure 5**. Importantly, some vessels that comprised these tumor supplying vessel subnetworks had either sibling or child vessels that did not service the tumor. These vessels were treated as part of the collection of vessels supplying healthy tissue.

We used a kernel density estimator (KDE) method to compare vascular branching scale factor distributions between tumor supplying and non-tumor supplying networks as reported in (Brummer et al., 2021). This approach can be interpreted as a multidimensional extension of the univariate Kolmogorov-Smirnov test (KS-test) (Duong et al., 2012). We compared two-dimensional distributions for the constrained scale factor pairs of $(\bar{\beta}, \Delta\beta)$ and $(\bar{\gamma}, \Delta\gamma)$, shown in **Figure 6**. The local KDE test identifies contours within the compared data that are uniquely responsible for driving differentiation between compared groups above a user defined threshold. This threshold has a natural translation into the conventional $p$-value of hypothesis testing (Duong, 2013). We chose to search for regions corresponding to the conventional $p$-value of 0.05, presented in **Figure 6C**. This technique is akin to layer-wise relevance propagation in deep learning algorithms (Montavon et al., 2019).

## 2.5. Metabolic Scaling From Vascular Measurements

By treating total metabolism of the supplied tissue as the sum of the metabolism of every terminal unit (e.g., cells and capillaries), $B_{tot} = B_{cap}N_{cap}$, and examining the total volume, $V_{tot}$, of the vascular network that supplies the volume of cells, one can exactly express the metabolic scaling exponent, $\theta$, as,

$$\theta = \begin{cases} \frac{\ln(2^N)}{\ln(2^N)+\ln(1-\nu^{N+1})-\ln(\nu^N(1-\nu))} & \text{for } \nu \neq 1 \\ \frac{\ln(2^N)}{\ln((N+1)2^N)-N\ln(\nu)} & \text{for } \nu \approx 1 \end{cases} \quad (4)$$

where $N$ represents the total number of generations within the network and $\nu$ represents the scaling of blood volume across a bifurcation and is equal to $\nu = 2\bar{\beta}^2\bar{\gamma} + 4\bar{\beta}\Delta\beta\Delta\gamma + 2\bar{\gamma}\Delta\beta^2$. The piecewise definition of Equation (4) is required due to the asymptotic limit when $\nu \approx 1$, or when the combined volume of two child branches is equal to that of their parent branch. Two important assumptions underlying Equation (4) are that the network is strictly bifurcating (two child vessels for every parent vessel) and that the degree of asymmetry in the network does not result in significant self-pruning of vessels at high generations. This second assumption has the interpretation that the number of vessels $N$ generations distal from a given parent vessel is approximately $2^N$. For detailed analyses of self-pruning in asymmetric pulmonary vascular networks see (Majumdar et al., 2005). An important conceptual interpretation of Equation (4), and metabolic scaling theory in general, is that it links the geometric distribution and delivery of blood supply to a given volume of metabolizing tissue.

To calculate metabolic scaling exponents for vascular trees using Equation (4), distributions of volumetric scale factors $\nu$ were first calculated for all bifurcations within the network. Geometric means for these distributions in $\nu$ were calculated to identify the average scaling of volume within each vascular tree. Estimates for the number of branching generations, $N$, within each vascular tree were determined from the number of terminal vessels, $N_{tips}$ within each tree using the expression $N = \log(N_{tips})/\log(2)$. As this expression produces non-integer values for the number of generations, $N$ was then rounded to the nearest integer. Examination of how the rounding of $N$ influences final estimates of $\theta$ demonstrated no significant change.

## 2.6. Metabolic and Gross Tumor Volume Scaling

An important prediction of metabolic scaling theory is a power-law relationship between the metabolic tumor volume ($MTV$) and the gross tumor volume ($GTV$) that incorporates the metabolic scaling exponent ($\theta$). The full derivation of this formula incorporates aspects of oxygen diffusion, vessel recruitment, and vascular branching arguments, and it can be found in the **Supplementary Material**. We present here the results of that argument,

$$MTV = V_0(GTV)^{\frac{2}{3\theta}} \quad (5)$$

where $V_0$ is a normalization constant. The 2/3 exponent reflects the fact that the metabolically active region of the tumor is an exterior shell, and thus a Euclidean surface-area-to-volume scaling occurs between this region and the total tumor volume. Equation (5) provides an important method to extract metabolic scaling exponents from PET-CT imaging, as the metabolically active tumor volume ($MTV$) is measured with both PET and CT modalities, while the gross tumor volume ($GTV$) is measured only with the CT modality. This is in addition to the scaling of maximum standard uptake value to metabolic tumor volume,

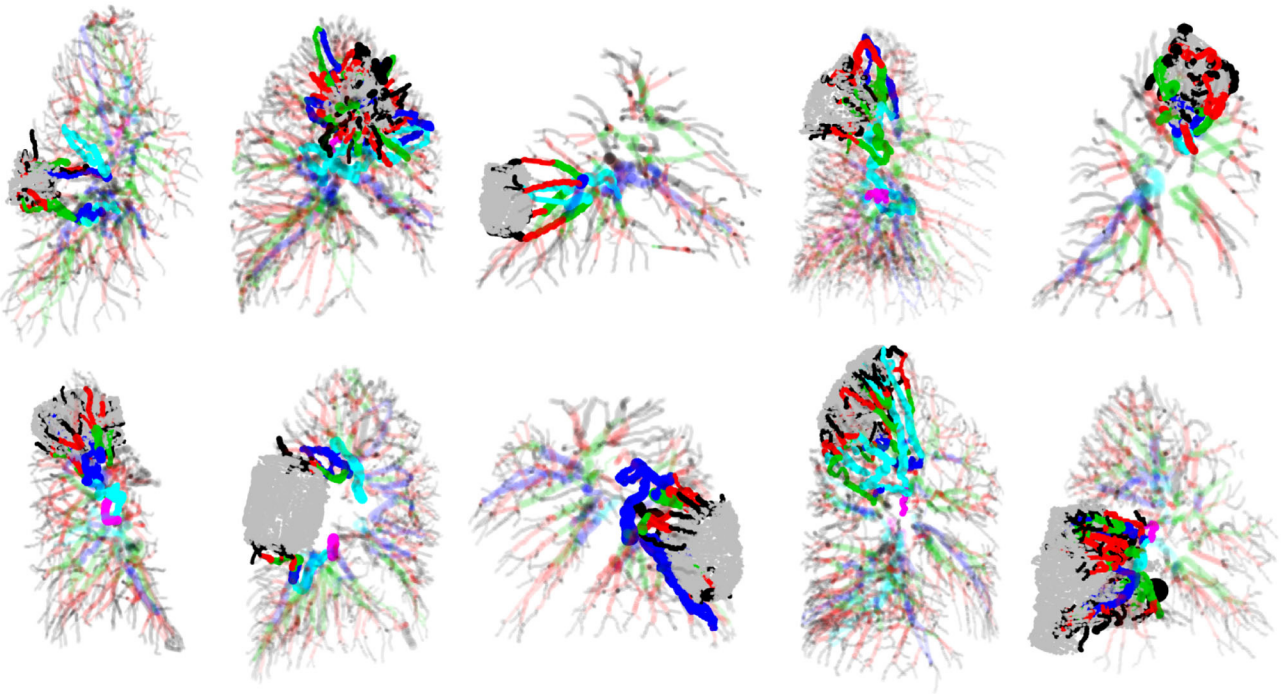

**FIGURE 5 |** Examples of tumors, the pulmonary vasculature in which they are embedded and supplied by, and remaining pulmonary vasculature of the lungs. Tumors are the rounded shapes colored in gray, and vessels are cylindrical shapes colored according to HS generation labeling. Tumor-supplying vasculature is drawn in full-color with zero transparency, while healthy-tissue supplying vasculature are drawn in color but with partial transparency.

given as

$$SUV_{max} = W_0(MTV)^{\theta} \tag{6}$$

where $W_0$ is a normalization constant. Both of Equations (5) and (6) are used to examine PET-CT data collected for this study, shown in **Figures 1**, **2**. Having dual measurements for the metabolic scaling exponent at the whole-tumor level gives added support when comparing these measurements against vascular based estimates using Equation (4).

# 3. RESULTS

## 3.1. Allometric Regressions

Examination of PET-CT imaging data shows clear allometric trends in **Figures 1**, **2**. For the larger dataset, we found that estimates of metabolic scaling exponents, $\theta$, based on $SUV_{max} \propto MTV^{\theta}$ are $\theta = 0.71 \pm 0.07$, with histologically specific values of $\theta = 0.73 \pm 0.09$ for adenocarcinomas (ADCs) and $\theta = 0.59 \pm 0.10$ for squamous cell carcinomas (SCCs) (see **Table 1**). These measurements are consistent with those from the subset of the PET-CT data from which vascular segmentations were examined. However, variation in $SUV_{max}$ is large enough that, with the smaller sample size for this latter subset, estimates for the metabolic scaling exponent $\theta$ are found to be less-significant for ADC and the combined data, and non-significant for SCCs. Estimates of $\theta$ based on the scaling of metabolic tumor volume and gross tumor volume, as in Equation (5), were found to be significant for both ADC ($\theta = 0.73 \pm 0.21$), SCC ($\theta = 0.62 \pm 0.37$), and the combined groups ($\theta = 0.70 \pm 0.17$). This finding indicates a potential robustness in image-based metabolic biomarkers that are distributed over the tumor volume, as opposed to those that are derived from the single brightest voxel.

## 3.2. Scale Factor Analysis

Scale factor analysis demonstrated significant differences between vasculature that supplies tumors and that which supplies healthy lung tissue. In **Figure 6A**, the joint distributions of the radial scale factors $(\bar{\beta}, \Delta\beta)$ for healthy-tissue supplying vasculature can be seen to adhere well to the area preservation constraint of Equation (2), particularly in the presence of asymmetry ($\Delta\beta \neq 0$). Whereas the tumor supplying vasculature tends to exhibit area increasing radial scaling. This is supported by the average scale factor values for $\bar{\beta}$ presented in **Table 2**, where $\bar{\beta} = 0.85 \pm 0.02$ for both ADC and SCC tumor vasculature, and $\bar{\beta} = 0.75 \pm 0.01$ for healthy-tissue vasculature. This is reinforced by the local KDE test in **Figure 6B**. Here the distinguishing regions of the different vessel category distributions are those corresponding precisely to area-preservation scaling for healthy tissue, and extreme area increasing scaling for the tumor vasculature. These differences have important physiological consequences related to fluid flow rates and blood volume supply that we explore in the Discussion.

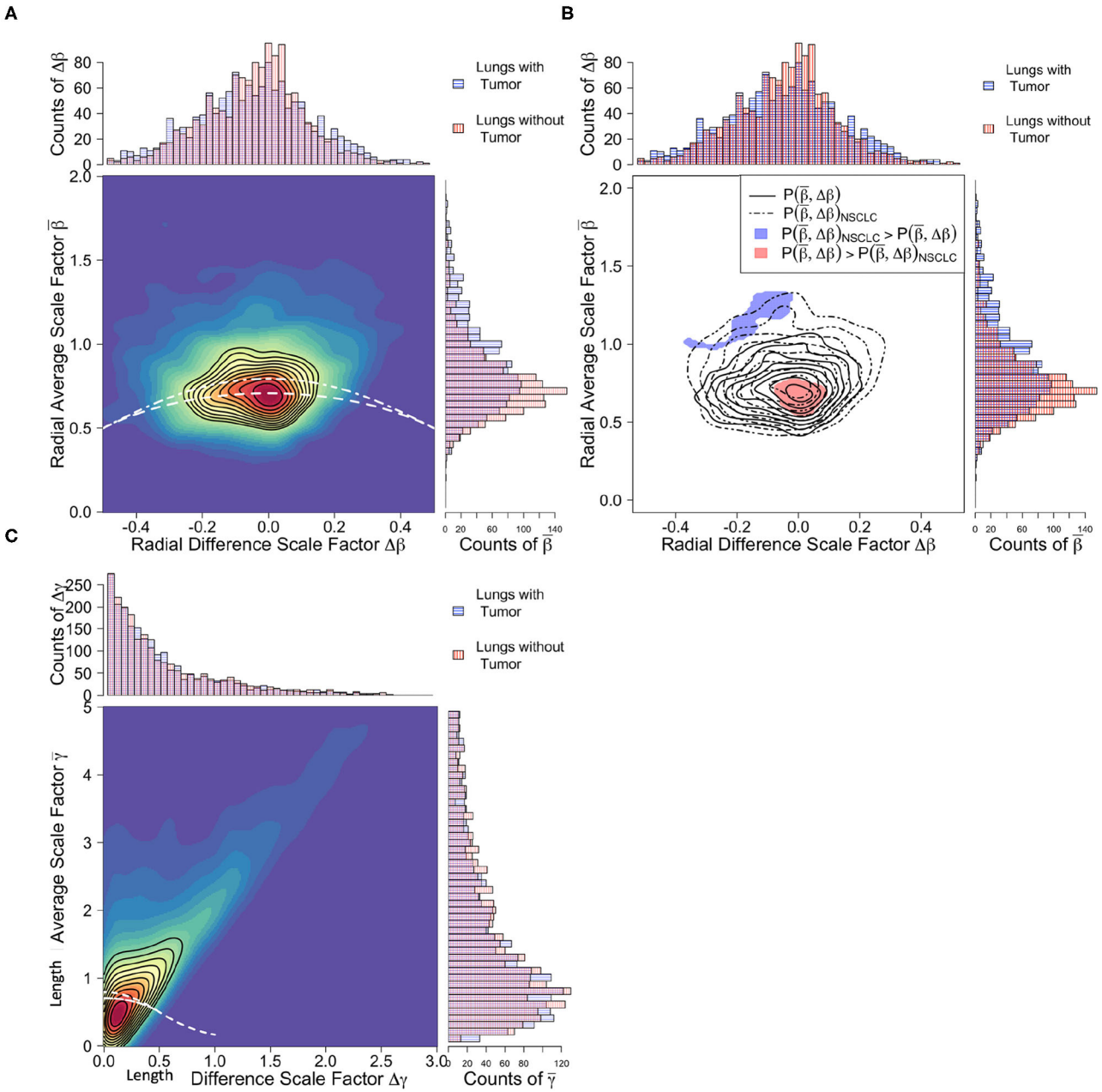


**FIGURE 6 |** Comparison of branching scale factors between tumor-supplying vessels (blue) and healthy tissue-supplying vessels (red). **(A)** Joint and marginal distributions for radial scale factors. White dashed lines correspond to predicted constraint equations for cubic, area-increasing scaling, Equation (3), (top line) and square, area-preserving scaling, Equation (2), (bottom line). Note that both categories of vessel groups closely follow area-preserving scaling, yet variation in the tumor-supplying vessel groups trend toward area-increasing scaling. **(B)** Significance contours identifying regions in the radial scale factor feature space responsible for differentiation. Unlike the radial scale factors, length scale factors **(C)** defined using canonical generation labeling do not result in significant differentiation between vessel groups.

Unlike the radial scale factors, no significant differences were observed between the tumor supplying and healthy tissue supplying vasculature in the average and difference length scale factor feature space $(\bar{\gamma}, \Delta\gamma)$, as seen in **Figure 6C** and **Table 2**. However, upon relabeling vessels using the Horton-Strahler topology, and remeasuring lengths and the Horton-Strahler length scale factor $\gamma_{HS}$, we found significant differences between the healthy vessel and tumor vessel populations (**Figure 4**). Specifically, we found that healthy vessels had an HS length scale factor of $\gamma_{HS} = 0.48 \pm 0.07$, while ADC HS length scale factors

averaged $\gamma_{HS} = 0.65 \pm 0.11$ and SCCs averaged $\gamma_{HS} = 0.73 \pm 0.32$. Theory does not presently exist to rigorously translate Horton-Strahler length scale factors into fractal dimensions of scaling. However, assuming that the fractal dimension can still be defined under the conventional manner as $D = -\log(2)/\log(\gamma_{HS})$, then one can still calculate the fractal dimension for a bifurcating system. In this case, the fractal dimension for the healthy tissue vasculature using Horton-Strahler labeling is $D = 0.94$, for ADCs is $D = 1.61$ and for SCCs is $D = 2.20$.

In addition to the conventional branching scale factors related to length and radius, we also analyzed the volumetric scale factor, $\nu$, the ratio of the volume of both child branches to the volume of the parent branch across a bifurcation. Expressed in terms of the radial and length average and difference scale factors, $\nu = 2\bar{\beta}^2\bar{\gamma} + 4\bar{\beta}\Delta\beta\Delta\gamma + 2\bar{\gamma}\Delta\beta^2$. We measured distributions of $\nu$ for every bifurcation across all vessel categories. We found significant differences in volumetric scaling between the two tumor categories of ADC and SCC and the healthy tissue supplying vasculature. Specifically, we measured the average volumetric scale factor for healthy tissue vasculature was $\nu = 2.05 \pm 0.27$, where for ADCs $\nu = 2.41 \pm 0.36$ and for SCCs $\nu = 2.52 \pm 0.34$. This pronounced difference in the volume of blood supplied by the vasculature is due in part to the observed difference in radial scaling, and has important consequences for metabolic scaling given the dependence on $\nu$ in Equation (4).

## 3.3. Vascular Based Estimates of Metabolic Scaling

Evaluation of Equation (4) to estimate metabolic scaling exponents from vascular branching resulted in average values for ADCs of $\theta = 0.85 \pm 0.03$ and for SCCs $\theta = 0.87 \pm 0.02$ (see **Table 1**). These values are within the 95% confidence intervals of the PET-CT imaging based estimates using the scaling of metabolic tumor volume ($MTV$) to gross tumor volume ($GTV$) from Equation (5). Importantly, the consistency of these two, simultaneous measurements marks a first-of-its-kind test of metabolic scaling theory.

Further analysis of the vascular based estimates of the metabolic scaling exponents is shown in **Figure 7**, where the dependence of the metabolic scaling exponent on volumetric scaling and total network generation is presented, with results for vasculature supplying ADCs, SCCs, and healthy lung tissue. We find that the tumors and healthy tissues cluster separately within the feature space of metabolic scaling exponent, $\theta$, number of network generations, $N$, and volumetric scale factor, $\nu$. Specifically, the ADCs have values of $N = 3.58 \pm 0.08$ and $\nu = 2.41 \pm 0.36$, the SCCs have values of $N = 4.03 \pm 0.07$ and $\nu = 2.52 \pm 0.34$, and the healthy tissue vascular networks have values of $N = 5.23 \pm 0.16$ and $\nu = 2.05 \pm 0.27$. These and other measured network values are reported in **Table 2**. The differences in the number of branching generations and volumetric scaling exist despite the fact that the tumors and healthy tissue vessel networks result in the same average values for the metabolic scaling exponent. This result serves to reinforce the potential value of vascular scale factors as imaging biomarkers for tumors.

# 4. DISCUSSION

In this work we demonstrate the potential for clinical cancer imaging to serve as a novel test of metabolic scaling theory. The nuclear and structural imaging modalities of positron emission tomography and X-ray computed tomography (PET-CT) provide a unique lens with which to examine metabolic scaling theory, with non-small cell lung cancer tumors serving as a model subject. We report several key findings as a result of this work, and summarize and discuss their implications. (i) We have conducted a first-of-its-kind simultaneous measurement of metabolic scaling. We utilize PET imaging to measure tumor glucose uptake as an estimate of metabolism via Equations (5) and (6), and CT imaging to measure the tumor-supplying vasculature that leads to vascular-based estimates of metabolic scaling via Equation (4). We report consistent measurements between these two approaches, a result that serves to validate the metabolic scaling theory of ecology. Furthermore, we highlight how growth models rooted in energetic partitioning connect these morphologic changes in tumor-supplying vasculature to tumor growth trajectories. (ii) We measure tumor-specific metabolic scaling exponents based on morphologic changes to the geometric scaling of the tumor-supplying vasculature. These structural changes in vascular scaling may serve as future imaging biomarkers to aid in disease detection, diagnosis, and stratification. (iii) We emphasize the opportunity for cancer to serve as a model subject to probe metabolic scaling theory at the onset of vascular development through the examination of small avascular tumors that transition to large vascularized tumors. (iv) Finally, we close by discussing several extensions and limitations of the work presented.

## 4.1. Simultaneous Measurements and Tumor Growth Trajectories

### 4.1.1. Simultaneous Measurements

Since the original inception of metabolic scaling theory in (West et al., 1997), simultaneous measurements of metabolic scaling have been elusive. However, these measurements are crucial for identifying strengths and weaknesses in the methods and theory of metabolic scaling, and for refining our understanding of metabolic scaling as a phenomenon (Price et al., 2012).

Our work presents a new and complementary perspective to the field of cancer biology which has recently seen a surge of interest in the scaling of tumor metabolism to tumor mass. Here, metabolic scaling is used as a quantitative framework for understanding the de-regulated growth of tumors facilitated by aerobic glycolysis, also known as the Warburg effect (Warburg, 1956; Vander Heiden et al., 2009). In these differing schools of thought, variation in metabolic scaling can be attributed to: variation in turnover rates from proliferative to necrotic states, resulting in transitions from linear to sub-linear scaling as in (Milotti et al., 2013); cell migration and competition as a driver of tumor subpopulations evolving from single to heterogenous states, resulting in transitions from sub-linear to super-linear scaling as in (Pérez-García et al., 2020); or sudden increases in oxygen supply levels as a result of angiogenesis, resulting in momentary accelerations in tumor growth and temporary

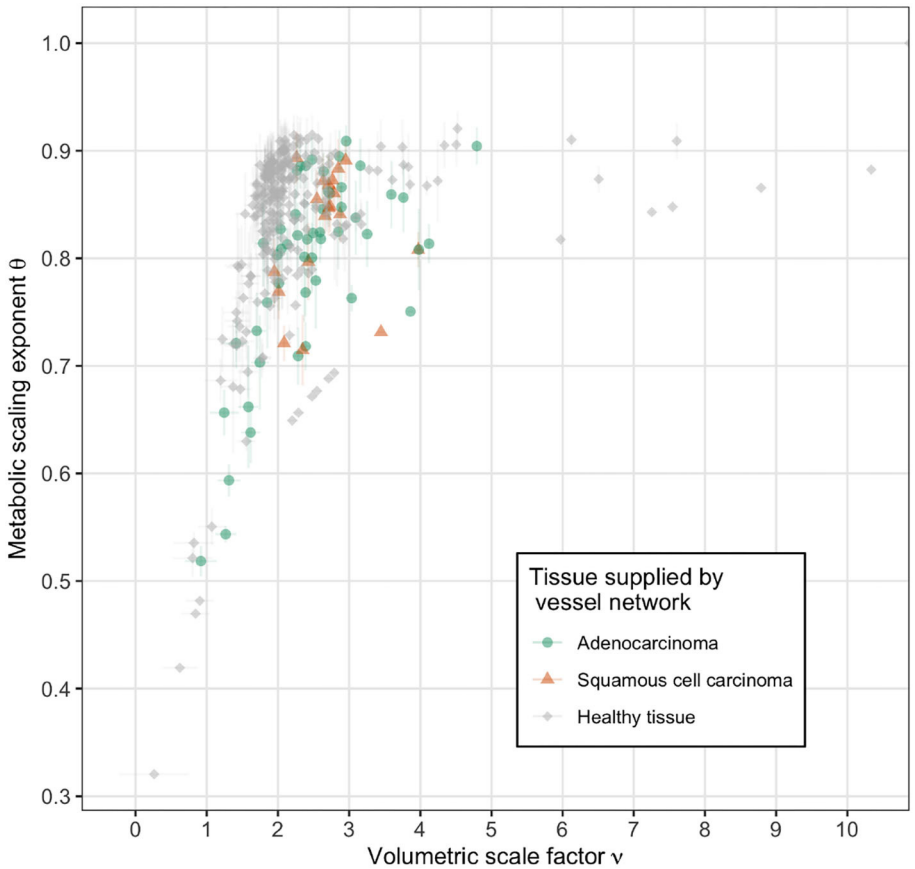


**FIGURE 7 |** Vascular based estimates of metabolic scaling exponents as a function of volumetric scale factor for tumor supplying vessel networks (adenocarcinoma in green and squamous cell carcinoma in orange) and healthy tissue supplying vessel networks (gray). The direction of increasing network size, as measured by generation number $N$, is into the upper left corner. Note the distinct clustering of the healthy tissue supplying networks along $\nu \approx 2$ and the tumor supplying networks along $\nu > 2$.

super-linear scaling as in (Azimzade et al., 2021). Here, we present a perspective that connects variation in metabolic scaling to variation in the geometric scaling of the tumor-supplying vasculature. While the many proposed mechanisms of variation in metabolic scaling offer complementary views of tumor growth and heterogeneity, they can also inform possible reasons for treatment failures.

Important benefits of simultaneous measurements are their ability to test the robustness of models and measurable features from experimental and clinical data. Although the two different regression methods of measuring metabolic scaling exponents in Equations (5) and (6) led to consistent results, both measurements rely heavily on the maximum standard update value ($SUV_{max}$) from the PET imaging. Despite the fact that $SUV_{max}$ will have little variation from differences in contouring, it can still vary from effects such as machine variability, patient physiology, and the partial volume effect. The latter of these is quite significant for metabolic scaling studies as it precludes many clinical PET imaging metrics from tumors smaller than 4 $cm^3$. While important work is being done in the realm of quantitative imaging biomarker discovery to standardize metrics (see Sullivan et al., 2015), we comment here on two other metrics of growing interest, total lesion activity ($TLA$) and glucose metabolic rate ($MR_{glu}$).

Recalling that $TLA$ is the summed product of voxel volume and $SUV_i$, or $TLA = \sum_i v_i SUV_i$, we argue caution should be made in using $TLA$ for examining metabolic scaling as it can introduce potentially spurious super-linear scaling. To see this, we highlight that if one calculates a volume-weighted average of $SUV$ as $\langle SUV \rangle_V = \sum_i v_i SUV_i / \sum_i v_i$, the definition of $TLA$ appears in the numerator. Solving for $TLA$ here results in, $TLA = V\langle SUV \rangle_V$. Thus, in comparing $TLA$ to $MTV$ as candidate metrics for a scaling phenomenon, a potential extra factor of volume can appear that may bias inferred scaling exponents toward the super-linear regime.

On the other hand, dynamic PET imaging may offer improved resolution of scaling phenomenon. Work by Visser et al. (2008) demonstrated that the combination of pharmacokinetic models of glucose uptake and dynamic PET imaging methods to measure the tumor glucose metabolic rate ($MR_{glu}$) result in systematic reduction in estimates of tumor volume when compared to static PET images. However, comparison of tumor volume estimates

from both static and dynamic PET imaging to tumor volumes measured after surgical resection show that both imaging metrics under-predicted tumor volume by as much as 50% (Meijer et al., 2017). Despite these varied differences, such work can still be valuable for metabolic scaling studies as over- or under-predicting tumor volume can be corrected as long as the deviations are systematic.

#### 4.1.2. Growth Trajectories

Interpretation of the value of the metabolic scaling exponent for tumors is most easily done through the context of growth models. Although the data considered in this study consists entirely of single point-in-time measurements, the metabolic scaling exponent can provide insight into tumor growth trajectories. Metabolic scaling theory can be extended to model the growth trajectory of an organ or organism using energetic partitioning. Derivations and analyses of the following model can be found in the literature (West et al., 2001; Guiot et al., 2006; Herman et al., 2011). Here we summarize the main points of an energetic tumor growth model, and in particular as it relates to the vascular supply network.

The growth of tumor mass over time can be expressed in the following differential equation,

$$\frac{dm}{dt} = \frac{B_0 m_c}{E_c} m^{\theta} - \frac{B_c}{E_c} m \tag{7}$$

where $m_c$ is the mass of an individual cell, $E_c$ is the energetic cost of cellular division, $B_c$ is the metabolic cost of cellular maintenance, and $B_0$ is the normalization constant for the metabolic scaling allometry. Equation (7) is derived by partitioning the total metabolic power available into two terms: one for cellular growth and the other for the cost of cellular maintenance. In Equation (7), the resulting growth term appears on the left-hand-side, while the terms for total metabolism minus the cost of maintenance appear on the right-hand-side. Historically, Equation (7) is also known as the Bertalanffy-Richards model of growth (Von Bertalanffy, 1957; Richards, 1959).

An important feature of Equation (7) is how the stability of the equilibrium mass, $m_{eq}$, changes with respect to the metabolic scaling exponent $\theta$. For super-linear metabolic scaling, where $\theta > 1$, the equilibrium mass is an unstable fixed point. Furthermore, if $m < m_{eq}$, the maintenance term in Equation (7) dominates the behavior and the system converges to $m_{eq} = 0$, whereas if $m > m_{eq}$ then the cost term is negligible, and the tumor mass grows without bound. For linear metabolic scaling, associated with laminar blood flow and where $\theta = 1$, Equation (7) reduces to that of exponential growth, with no stable equilibrium. Finally, for sub-linear scaling, where $\theta < 1$, the equilibrium mass is a stable fixed point.

The combination of Equations (4) and (7) highlights how patterns in vascular development during tumor angiogenesis can determine a growth trajectory (this is in addition to the histologically specific values of $m_c, E_c, B_c$, and $B_0$. In particular, as a tumor neoplasm develops, it first exists in the linear metabolic scaling regime with no equilibrium state and exhibits runaway growth. Once the tumor begins secreting angiogenic factors, new vasculature develops to supply the tumor with blood, subsequently reshaping the local vascular branching geometry and driving the tumor into the sub-linear metabolic scaling regime with a stable equilibrium. Thus, in the context of metabolic scaling theory, the geometric scaling of the tumor vasculature can serve as a measurable bifurcation parameter of the tumor growth trajectory.

The vascular-based tuning of growth demonstrates the importance for future measurements of both metabolic scaling exponents and tumor microenvironment variables extracted from pathology samples and/or altogether new metabolic radio-tracers to determine the overall tumor growth trajectory (Momcilovic et al., 2019). These efforts are especially important in understanding variation in tumor growth due to differences in tumor histology (e.g., squamous cell carcinoma and adenocarcinoma) and heterogeneity.

### 4.2. Vascular Morphogenesis

Our work in examining metabolic scaling in tumors relies heavily on measurements of the pulmonary vascular-network that is supplying the tumors with blood. We propose a procedure for identifying the tumor-supplying vessels as those that penetrate the segmented tumor contours, and a routine for identifying the vascular tree in which these vessels are connected. We subsequently demonstrate that these tumor-supplying vascular trees exhibit markedly different radial scaling than the healthy lung tissue-supplying vascular networks (**Figure 6**). These differences have important connections to volumetric blood-flow rates.

There are two potential physiological consequences for the observed differences in radial scaling. Conservation of fluid flow dictates that area increasing scaling will result in a slowing of the blood flow from parent to child vessels across a bifurcation. Large tumors will necessarily attach to large diameter vessels, which diminishes the number of branching generations that would normally be present to slow the flow of blood. Thus, vessel widening may be viewed as a compensatory mechanism to facilitate the slowing down of blood flow in the absence of a sufficient number of branching generations.

The second consequence for increased radial scaling is increasing the total blood volume delivered per unit time. This is supported by the observed increase in volumetric scaling for tumor-supplying vessels over healthy tissue-supplying vessels (**Figure 7** and **Table 2**). As a tumor grows it places an increasing demand on nutrient supply in terms of blood volume. This can be accomplished by increasing either the vessel length or vessel radius. However, an increase in vessel length can only occur through the process of growing wholly new vessels, or by pruning existing vessels at a branch point, whereas increasing vessel radius can be achieved in any existing vessel. Furthermore, as changes in vessel volume are constant with respect to length and linear with respect to radius, the benefit of increasing radius is two-fold. That is, doubling the length only doubles the volume, but doubling the radius will quadruple the volume.

## 4.3. Metabolic Scaling in Avascular Systems

Finally, this work highlights important opportunities for insights and perspectives from cancer biology to inform ecology and evolutionary biology. Although applications of metabolic scaling in ecology and evolutionary biology permeate a myriad of systems (e.g., food webs, predator-prey interactions, forrest demography, and species-area distributions) a central hallmark of metabolic scaling theory is the reliance on a well-defined vascular network distributing resources. Thus, in systems absent of resource distribution networks, deviations from metabolic scaling predictions can be challenging to interpret.

The biological processes of tumor growth and angiogenesis represent a unique opportunity to study metabolic scaling theory beyond the vascular regime. Many conventional tumor growth and angiogenesis models are cast as reaction-diffusion processes that highlight different phenomena operating at different scales (Hormuth et al., 2021). Cellular-scale models incorporate any number of terms specific to cellular growth, death, interactions, and importantly include cellular motion due to diffusion, advection and chemical attraction (Frieboes et al., 2010). Tissue-scale models balance the demands of fluid transport within vascular networks, fluid flux through vessel walls, and interstitial flow that links the embedding tissues with the supplying vessels (Wu et al., 2020).

Metabolic scaling theory may serve to bridge these phenomenological scales by linking geometric patterns of tissue vasculature to the metabolic demands of cellular processes. Alternatively, framing the cell and tissue scale processes in the context of metabolic scaling theory may help to inform the modeling of other biological systems conventional to ecology and evolutionary biology. For example, models of cellular diffusion bear much resemblance to those of species aggregation and migration, and could inform recent efforts in landscape disturbance ecology (Harte et al., 2021). Similarly, the interaction between tumor driven angiogenesis and changes in the tumor microenvironment may guide studies on the interaction between environmental drivers and biomechanical limits to cellular evolution (Malerba and Marshall, 2021).

## 4.4. Extensions and Limitations

Here we outline several extensions and limitations of this work. These span: improvements in technical analyses and model approaches; the focus of pulmonary arterial networks over bronchial arterial networks; applications in tumor directed chemotherapy, embolization, and malignancy determination; and the general study of other cancers.

### 4.4.1. Small Lesions and Partial Volume Limitations

A challenge of the current study is the inability to accurately apply metabolic scaling theory to the study of small lesions, here defined as having a tumor volume less than 4 $cm^3$. This is a consequence of the partial volume effect, an imaging artifact in which objects near the resolution limit may appear larger than they actually are (Soret et al., 2007). The partial volume effect can skew measurements of tumor and vessel volumes extracted from CT imaging, in addition to metabolic measurements from PET imaging. Important work has been done to quantify the size of the partial volume effect in PET imaging by using imaging phantoms (materials designed with known radioactivities) (Kinahan et al., 2009), as well as to define analysis protocols to correct for the partial volume effect based on background PET measurements in the vicinity of a lesion (Salavati et al., 2017). Regarding CT imaging, recent studies of variation in image acquisition (dose and resolution) and reconstruction methods are providing important insight into the source and nature of variation in lesion detection and quantification (Lo et al., 2016). Finally, recent work leveraging mathematical theorems of vessel shape and geometry have identified systematic procedures for subsampling vessel image data to resolutions beyond the imaging modality limit (Brummer et al., 2021). Thus, while small lesions were omitted from this work, future efforts have multiple avenues available for their inclusion.

### 4.4.2. Horton-Strahler Corrections to Vascular Branching Architecture

The biased differences between the PET-CT derived estimates for the metabolic scaling exponents and the vascular based estimates can be partially resolved with careful examination of the length scale factors of the vascular networks. A common finding in measuring length scale factors is the heavy-tailed structure of their distributions (Newberry et al., 2015; Tekin et al., 2016; Brummer et al., 2021). This results in large distribution means for length scale factors ($\langle\bar{\gamma}\rangle > 1$), which can subsequently increase the estimate of the metabolic scaling exponent. A benefit of the Horton-Strahler labeling scheme is that it systematically lowers the estimates for length scaling and results in more biophysically realistic estimates of the fractal dimension for the network (**Figure 4E** and **Table 2**). Furthermore, we can approximate the impact of the length scaling bias on estimates of the metabolic scaling exponent by utilizing the Horton-Strahler length scale factor and the fact that the vascular networks studied exhibit radial symmetry.

An exact derivation of Equation (4) for the Horton-Strahler topology remains elusive, primarily due to the challenges of bridging the formalisms for asymmetrically branching networks. However, our measurements show that the vascular systems exhibit radial symmetry, $\langle\Delta\beta\rangle \approx 0$ (see **Figure 6** and **Table 2**). Thus, the definition of the volume scale factor reduces to $\nu \approx 2\bar{\beta}^2\gamma_{HS}$, where we have substituted $\bar{\gamma}$ with $\gamma_{HS}$ under a symmetric approximation. Evaluating Equation (4) with this approximate form of $\nu$ and values taken from **Table 2** results in estimates of the metabolic scaling exponent of $\theta_{HS} = 0.60$ for ADCs and $\theta_{HS} = 0.65$ for SCCs, values that are intriguingly closer to those extracted from the PET-CT allometric scaling measurements. This suggests the HS topology may be a more appropriate labeling scheme for these types of vasculature (**Table 1**).

### 4.4.3. Pulmonary vs. Bronchial (Systemic) Arterial Networks

A unique feature of the lung is that it possesses a dual arterial blood supply. The pulmonary arterial trees are responsible for oxygenating the blood supply and dispelling byproducts of systemic cellular respiration, while the bronchial (or systemic)

arterial trees provide oxygenated blood specifically to lung tissue for their own cellular respiration. This dual blood supply feature of the lungs is also shared with lung tumors (Milne, 1967), and resolving the extent to which the different vascular networks are involved in tumor initiation, growth, histology, and metastasization is an active field of research (Nguyen-Kim et al., 2015; Eldridge et al., 2016; Deng et al., 2020).

Importantly for this study, the diameters of the vessels in the bronchial arterial tree are an order of magnitude smaller than those found in the pulmonary arterial tree. This results in many of the bronchial vessels not being detected in typical clinical CT imaging devices and likely being absent from our study. There exist methods to simultaneously measure both pulmonary and bronchial arterial networks using carefully designed perfusion CT imaging. However, these methods are technologically demanding and require exceptionally large doses of radiation to be given to patients, precluding their widespread adoption (Yuan et al., 2012; Nguyen-Kim et al., 2015).

The absence of bronchial vascular networks from our study may contribute to the observed difference between the allometric-based and vascular-branching-based measurements of metabolic scaling exponents (**Table 1**). The exact size of the contribution of the bronchial networks to the total tumor metabolism is not currently known. However, current understanding suggests as tumors increase in size they begin to undergo hypoxia internally and subsequently develop necrotic cores. As a result, vessel recruitment through either angiogenesis or cooption of the pulmonary arterial vessels can occur at the tumor boundary to facilitate an increase in tumor blood supply. Thus, we presume that by focusing this study to larger tumors of clinical staging II-IV, the reliance on bronchial arterial supply is minimized.

#### 4.4.4. Tumor Directed Therapies and Embolization

Tumor directed therapies—ones that try to localize treatment more to the local tumor and not the whole body—may be greatly informed by distinguishing between bronchial and pulmonary tumor vascular supply, providing a major motivator for future studies in that direction. Expansive knowledge of the vascular supply to hepatic tumors has led to a multitude of standard treatment options that combine vascular embolization with any of radio-, chemo-, or immunotherapy (Erinjeri et al., 2019). These methods provide localized tumor directed treatment and alleviate many of the complicating side-effects associated with systemic (whole-body) approaches or surgical intervention. In the treatment of lung cancer, transpulmonary chemoembolization has proven successful as an interventional technique, yet has questionable impact on overall patient survival (Lindemayr et al., 2007; Vogl et al., 2013). We propose our framework of coupling tumor vascular supply to tumor metabolism as a method for screening for patients that are likely to respond to tumor directed therapies. Specifically, tumors whose vascular supply is dominated by the pulmonary arterial vasculature may be good candidates for these therapies as the likelihood of treatment escape through the bronchial arterial supply should be minimal.

#### 4.4.5. Malignancy Determination in Lung Cancer

The methods and results presented here have potential to serve as biomarkers of tumor malignancy. Previous work by others has demonstrated the ability of blood vessel branching metrics to serve as indicators of tumor malignancy. In a radiomics-inspired study, blood vessel volume was identified as an imaging biomarker that could distinguish between adenocarcinomas and granulomas in the networks supplying and surrounding the nodules (Alilou et al., 2018). Blood vessel volume would be most directly related to our volumetric scale factor, $\nu$, which we found to indicate significant differences between blood vessel networks supplying healthy and tumor burdened lungs (**Table 2**). Furthermore, another study identified blood vessel diameter as being indicative of malignant vs. benign classification in a patient cohort with comorbidities of chronic obstructive pulmonary disorder (e.g., emphysema) (Wang et al., 2017). Blood vessel diameter constitutes our radial average and difference scale factors, $\bar{\beta}$ and $\Delta\gamma$, which also demonstrated significant differences between healthy and tumor burdened pulmonary vessels (**Table 2**). Finally, our measurements of vessel length scaling, specifically the average length scale factor, $\bar{\gamma}$, and the Horton-Strahler length scale factor, $\gamma_{HS}$, identified modest differences between adenocarcinomas and squamous cell carcinomas, suggesting that histologically-based differences in tumor-supplying vasculature may exist (**Table 2**). These results motivate future work to better quantify the diagnostic potential of blood vessel biomarkers both for malignancy determination and histological stratification.

#### 4.4.6. General Study of Other Cancers

While this work focused on the application of metabolic scaling theory to the study of non-small cell lung carcinoma and PET-CT imaging, it should be applicable to other cancers and imaging modalities. Recent work by Pérez-García et al. (2020) has demonstrated the existence of metabolic allometries in brain, lung, breast, rectal, and head and neck cancers. The challenge that persists is accurate segmentation of the supplying vasculature, and more so, the vasculature that comprises the tumor itself. This is a challenge at the intersection of imaging modality and computer vision. We chose to focus on the lung in order to minimize the presence of background tissue that may complicate accurate vessel segmentation using high dose CT. However, ongoing efforts that utilize vessel segmentation to guide therapeutic interventions and track treatment response in liver and breast cancer using diffusion-weighted contrast-enhanced magnetic resonance imaging (see Marčan et al., 2015; Wu et al., 2020) may benefit from metabolic scaling theory, or even contribute to its development. In fact, the highly vascularized nature of liver tumors offers a unique opportunity to test the assumption that tumor-supplying vessels are a sufficient proxy for tumor-comprising vessels.

## 5. CONCLUSION

We present a first-of-its-kind test of metabolic scaling theory. Leveraging clinical PET-CT imaging across a cohort of patients,

and computer vision methods to extract pulmonary vascular segmentation, we simultaneously measure metabolic scaling exponents from allometric regressions on tumor metabolism and mass as well as geometric models of the vascular branching architecture. The consistency of these measurements supports the framework of metabolic scaling theory, and introduces new opportunities for imaging biomarkers in the detection, diagnostics, and tracking of non-small cell lung carcinoma. Specifically, we find that the pulmonary vascular networks that supply tumors with blood exhibit area-increasing radial scaling with essential physiological consequences. This scaling facilitates a slowing of blood flow and an increase in total blood volume delivered. In combination with measurements of tumor cell proliferation from histological studies, these vascular imaging features can be utilized for the prediction of tumor growth. Additionally, this work highlights unique opportunities to further develop and test the metabolic scaling theory of ecology in avascular systems.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found at the Cancer Imaging Archive: https://www.cancerimagingarchive.net/ at the repository titled "Radiogenomics". Data collected and generated are available on a Github repository https://github.com/alexbbrummer/NSCLC_metabolic_scaling.

## ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

ABB and VMS designed the study. ABB assembled the data, performed all the analyses, and wrote the first draft of the manuscript. Both authors have discussed the results and contributed extensively to the final manuscript.

## FUNDING

ABB was supported in part by the National Science Foundation (grant 1254159).

## ACKNOWLEDGMENTS

The authors would like to acknowledge the following individuals who contributed data to this study as well as comments on the initial draft: Sarah Mattonen (Mattonen et al., 2019), Yoshihasa Shimada and Yojiro Makino (Furumoto et al., 2018), David Chardin and Olivier Humbert (Chardin et al., 2020), and Jesús Bosque Martínez and Víctor Manuel Pérez García (Pérez-García et al., 2020). Furthermore, the authors would like to acknowledge the following individuals for insightful discussions during the development of this work and manuscript: Mary Sehl, David Shackelford, Russell Rockne, Michael McNitt-Gray, Aaron Lisberg, Eric Deeds, and Vikram Adhikarla.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.691830/full#supplementary-material

## REFERENCES

Aerts, H. J., Velazquez, E. R., Leijenaar, R. T., Parmar, C., Grossmann, P., Carvalho, S., et al. (2014). Decoding tumour phenotype by noninvasive imaging using a quantitative radiomics approach. *Nat. Commun.* 5:4006. doi: 10.1038/ncomms5644

Alilou, M., Orooji, M., Beig, N., Prasanna, P., Rajiah, P., Donatelli, C., et al. (2018). Quantitative vessel tortuosity: a potential CT imaging biomarker for distinguishing lung granulomas from adenocarcinomas. *Sci. Rep.* 8:15290. doi: 10.1038/s41598-018-33473-0

Apte, R. S., Chen, D. S., and Ferrara, N. (2019). Vegf in signaling and disease: beyond discovery and development. *Cell* 176, 1248–1264. doi: 10.1016/j.cell.2019.01.021

Ardila, D., Kiraly, A. P., Bharadwaj, S., Choi, B., Reicher, J. J., Peng, L., et al. (2019). End-to-end lung cancer screening with three-dimensional deep learning on low-dose chest computed tomography. *Nat. Med.* 25, 954–961. doi: 10.1038/s41591-019-0447-x

Azimzade, Y., Saberi, A. A., and Gatenby, R. A. (2021). Superlinear growth reveals the allee effect in tumors. *Phys. Rev. E* 103:042405. doi: 10.1103/PhysRevE.103.042405

Bailey, D. L., Maisey, M. N., Townsend, D. W., and Valk, P. E. (2005). *Positron Emission Tomography: Basic Sciences*, Vol. 2. London: Springer.

Banavar, J. R., Moses, M. E., Brown, J. H., Damuth, J., Rinaldo, A., Sibly, R. M., et al. (2010). A general basis for quarter-power scaling in animals. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15816–15820. doi: 10.1073/pnas.1009974107

Barneche, D. R., Robertson, D. R., White, C. R., and Marshall, D. J. (2018). Fish reproductive-energy output increases disproportionately with body size. *Science* 360, 642–645. doi: 10.1126/science.aao6868

Barnsley, M. F. (2012). *Fractals Everywhere.* Boston: Academic Press.

Bejan, A. (2001). The tree of convective heat streams: its thermal insulation function and the predicted 3/4-power relation between body heat loss and body size. *Int. J. Heat Mass Transfer* 44, 699–704. doi: 10.1016/S0017-9310(00)00138-1

Bentley, L. P., Stegen, J. C., Savage, V. M., Smith, D. D., von Allmen, E. I., Sperry, J. S., et al. (2013). An empirical assessment of tree branching networks and implications for plant allometric scaling models. *Ecol. Lett.* 16, 1069–1078. doi: 10.1111/ele.12127

Bettencourt, L. M., Lobo, J., Helbing, D., Kühnert, C., and West, G. B. (2007). Growth, innovation, scaling, and the pace of life in cities. *Proc. Natl. Acad. Sci. U.S.A.* 104, 7301–7306. doi: 10.1073/pnas.0610172104

Brose, U., Williams, R. J., and Martinez, N. D. (2006). Allometric scaling enhances stability in complex food webs. *Ecol. Lett.* 9, 1228–1236. doi: 10.1111/j.1461-0248.2006.00978.x

Brummer, A. B., Hunt, D., and Savage, V. (2021). Improving blood vessel tortuosity measurements via highly sampled numerical integration of the Frenet-Serret equations. *IEEE Trans. Med. Imaging* 40, 297–309. doi: 10.1109/TMI.2020.3025467

Brummer, A. B., Lymperopoulos, P., Shen, J., Tekin, E., Bentley, L. P., Buzzard, V., et al. (2021). Branching principles of animal and plant networks identified by combining extensive data, machine learning and modelling. *J. R. Soc. Interface* 18:20200624. doi: 10.1098/rsif.2020.0624

Brummer, A. B., Savage, V. M., and Enquist, B. J. (2017). A general model for metabolic scaling in self-similar asymmetric networks. *PLoS Comput. Biol.* 13:e1005394. doi: 10.1371/journal.pcbi.1005394

Chardin, D., Paquet, M., Schiappa, R., Darcourt, J., Bailleux, C., Poudenx, M., et al. (2020). Baseline metabolic tumor volume as a strong predictive and prognostic biomarker in patients with non-small cell lung cancer treated with pd1 inhibitors: a prospective study. *J. Immunother. Cancer* 8:e000645. doi: 10.1136/jitc-2020-000645

Clark, K., Vendt, B., Smith, K., Freymann, J., Kirby, J., Koppel, P., et al. (2013). The cancer imaging archive (TCIA): maintaining and operating a public information repository. *J. Digital Imaging* 26, 1045–1057. doi: 10.1007/s10278-013-9622-7

DeLong, J. P., Okie, J. G., Moses, M. E., Sibly, R. M., and Brown, J. H. (2010). Shifts in metabolic scaling, production, and efficiency across major evolutionary transitions of life. *Proc. Natl. Acad. Sci. U.S.A.* 107, 12941–12945. doi: 10.1073/pnas.1007783107

Deng, L., Tang, H., Qiang, J., Wang, J., and Xiao, S. (2020). Blood supply of early lung adenocarcinomas in mice and the tumor-supplying vessel relationship: a micro-CT angiography study. *Cancer Prevent. Res.* 13, 989–996. doi: 10.1158/1940-6207.CAPR-20-0036

Dodds, P. S. (2010). Optimal form of branching supply and collection networks. *Phys. Rev. Lett.* 104:048702. doi: 10.1103/PhysRevLett.104.048702

D'Sa, R., Lewis, K., Pereira, J., Thomas, V., Chavan, D., et al. (2019). "Comparative analysis of lung segmentation," in *Proceedings of International Conference on Communication and Information Processing (ICCIP)*. doi: 10.2139/ssrn.3424473

Duong, T. (2013). Local significant differences from nonparametric two-sample tests. *J. Nonparametric Stat.* 25, 635–645. doi: 10.1080/10485252.2013.810217

Duong, T., Goud, B., and Schauer, K. (2012). Closed-form density-based framework for automatic detection of cellular morphology changes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 8382–8387. doi: 10.1073/pnas.1117796109

Eldridge, L., Moldobaeva, A., Zhong, Q., Jenkins, J., Snyder, M., Brown, R. H., et al. (2016). Bronchial artery angiogenesis drives lung tumor growth. *Cancer Res.* 76, 5962–5969. doi: 10.1158/0008-5472.CAN-16-1131

Eloy, C., Fournier, M., Lacointe, A., and Moulia, B. (2017). Wind loads and competition for light sculpt trees into self-similar structures. *Nat. Commun.* 8:1014. doi: 10.1038/s41467-017-00995-6

Enquist, B. J., Brown, J. H., and West, G. B. (1998). Allometric scaling of plant energetics and population density. *Nature* 395, 163–165. doi: 10.1038/25977

Enquist, B. J., West, G. B., and Brown, J. H. (2009). Extensions and evaluations of a general quantitative theory of forest structure and dynamics. *Proc. Natl. Acad. Sci. U.S.A.* 106, 7046–7051. doi: 10.1073/pnas.0812303106

Erinjeri, J. P., Fine, G. C., Adema, G. J., Ahmed, M., Chapiro, J., Den Brok, M., et al. (2019). Immunotherapy and the interventional oncologist: challenges and opportunities—a society of interventional oncology white paper. *Radiology* 292, 25–34. doi: 10.1148/radiol.2019182326

Frieboes, H. B., Jin, F., Chuang, Y.-L., Wise, S. M., Lowengrub, J. S., and Cristini, V. (2010). Three-dimensional multispecies nonlinear tumor growth—II: tumor invasion and angiogenesis. *J. Theoret. Biol.* 264, 1254–1278. doi: 10.1016/j.jtbi.2010.02.036

Furumoto, H., Shimada, Y., Imai, K., Maehara, S., Maeda, J., Hagiwara, M., et al. (2018). Prognostic impact of the integration of volumetric quantification of the solid part of the tumor on 3DCT and FDG-PET imaging in clinical stage IA adenocarcinoma of the lung. *Lung Cancer* 121, 91–96. doi: 10.1016/j.lungcan.2018.05.001

Gevaert, O., Xu, J., Hoang, C. D., Leung, A. N., Quon, A., Rubin, D. L., et al. (2012). Non-small cell lung cancer: identifying prognostic imaging biomarkers by leveraging public gene expression microarray data-methods and preliminary results. *Radiology* 264, 387–396. doi: 10.1148/radiol.12111607

Guiot, C., Degiorgis, P. G., Delsanto, P. P., Gabriele, P., and Deisboeck, T. S. (2003). Does tumor growth follow a "universal law"? *J. Theoret. Biol.* 225, 147–151. doi: 10.1016/S0022-5193(03)00221-2

Guiot, C., Delsanto, P. P., Carpinteri, A., Pugno, N., Mansury, Y., and Deisboeck, T. S. (2006). The dynamic evolution of the power exponent in a universal growth model of tumors. *J. Theoret. Biol.* 240, 459–463. doi: 10.1016/j.jtbi.2005.10.006

Harte, J., and Newman, E. A. (2014). Maximum information entropy: a foundation for ecological theory. *Trends Ecol. Evol.* 29, 384–389. doi: 10.1016/j.tree.2014.04.009

Harte, J., Umemura, K., and Brush, M. (2021). Dynamete: a hybrid maxent-plus-mechanism theory of dynamic macroecology. *Ecol. Lett.* 24, 935–949. doi: 10.1111/ele.13714

Hatton, I. A., McCann, K. S., Fryxell, J. M., Davies, T. J., Smerlak, M., Sinclair, A. R., et al. (2015). The predator-prey power law: biomass scaling across terrestrial and aquatic biomes. *Science* 349:aac6284. doi: 10.1126/science.aac6284

Herman, A. B., Savage, V. M., and West, G. B. (2011). A quantitative theory of solid tumor growth, metabolic rate and vascularization. *PLoS ONE* 6:e22973. doi: 10.1371/journal.pone.0022973

Hormuth, D. A., Phillips, C. M., Wu, C., Lima, E. A. B. F., Lorenzo, G., Jha, P. K., et al. (2021). Biologically-based mathematical modeling of tumor vasculature and angiogenesis via time-resolved imaging data. *Cancers* 13:3008. doi: 10.3390/cancers13123008

Horton, R. E. (1945). Erosional development of streams and their drainage basins; hydrophysical approach to quantitative morphology. *Geol. Soc. Am. Bull.* 56, 275–370. doi: 10.1130/0016-7606(1945)56[275:EDOSAT]2.0.CO;2

Huang, Q., Hu, X., He, W., Zhao, Y., Hao, S., Wu, Q., et al. (2018). Fluid shear stress and tumor metastasis. *Am. J. Cancer Res.* 8:763.

Huo, Y., and Kassab, G. S. (2009a). A scaling law of vascular volume. *Biophys. J.* 96, 347–353. doi: 10.1016/j.bpj.2008.09.039

Huo, Y., and Kassab, G. S. (2009b). The scaling of blood flow resistance: from a single vessel to the entire distal tree. *Biophys. J.* 96, 339–346. doi: 10.1016/j.bpj.2008.09.038

Jain, R. K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 307, 58–62. doi: 10.1126/science.1104819

Jin, Y., Fayad, L. M., and Laine, A. F. (2001). "Contrast enhancement by multiscale adaptive histogram equalization," in *Proc. SPIE 4478, Wavelets: Applications in Signal and Image Processing IX*. doi: 10.1117/12.449705

Kinahan, P. E., Doot, R. K., Wanner-Roybal, M., Bidaut, L. M., Armato III, S. G., Meyer, C. R., et al. (2009). PET/CT assessment of response to therapy: tumor change measurement, truth data and error. *Transl. Oncol.* 2, 223–230. doi: 10.1593/tlo.09223

Kinahan, P. E., and Fletcher, J. W. (2010). Positron emission tomography-computed tomography standardized uptake values in clinical practice and assessing response to therapy. *Semin. Ultrasound CT MRI* 31, 496–505. doi: 10.1053/j.sult.2010.10.001

Kleiber, M. (1932). Body size and metabolism. *Hilgardia* 6, 315–353. doi: 10.3733/hilg.v06n11p315

Lambin, P., Leijenaar, R. T., Deist, T. M., Peerlings, J., De Jong, E. E., Van Timmeren, J., et al. (2017). Radiomics: the bridge between medical imaging and personalized medicine. *Nat. Rev. Clin. Oncol.* 14:749. doi: 10.1038/nrclinonc.2017.141

Lau, A., Martius, C., Bartholomeus, H., Shenkin, A., Jackson, T., Malhi, Y., et al. (2019). Estimating architecture-based metabolic scaling exponents of tropical trees using terrestrial lidar and 3D modelling. *Forest Ecol. Manage.* 439, 132–145. doi: 10.1016/j.foreco.2019.02.019

Lindemayr, S., Lehnert, T., Korkusuz, H., Hammerstingl, R., and Vogl, T. J. (2007). Transpulmonary chemoembolization: a novel approach for the treatment of unresectable lung tumors. *Tech. Vasc. Intervent. Radiol.* 10, 114–119. doi: 10.1053/j.tvir.2007.09.010

Lo, P., Young, S., Kim, H., Brown, M., and McNitt-Gray, M. (2016). Variability in CT lung-nodule quantification: effects of dose reduction and reconstruction methods on density and texture based features. *Med. Phys.* 43, 4854–4865. doi: 10.1118/1.4954845

Majumdar, A., Alencar, A. M., Buldyrev, S. V., Hantos, Z., Lutchen, K. R., Stanley, H. E., et al. (2005). Relating airway diameter distributions to regular branching asymmetry in the lung. *Phys. Rev. Lett.* 95:168101. doi: 10.1103/PhysRevLett.95.168101

Malerba, M. E., and Marshall, D. J. (2021). Larger cells have relatively smaller nuclei across the tree of life. *Evol. Lett.* 5, 306–314. doi: 10.1002/evl3.243

Mandelbrot, B. B. (1982). *The Fractal Geometry of Nature*. San Francisco, CA: W. H. Freeman and Co.

Marčan, M., Kos, B., and Miklavčič, D. (2015). Effect of blood vessel segmentation on the outcome of electroporation-based treatments of liver tumors. *PLOS ONE* 10:e0125591. doi: 10.1371/journal.pone.0125591

Mattonen, S. A., Davidzon, G. A., Bakr, S., Echegaray, S., Leung, A. N., Vasanawala, M., et al. (2019). [18f] fdg positron emission tomography (PET) tumor and penumbra imaging features predict recurrence in non–small cell lung cancer. *Tomography* 5:145. doi: 10.18383/j.tom.2018.00026

Meijer, T. W., de Geus-Oei, L.-F., Visser, E. P., Oyen, W. J., Looijen-Salamon, M. G., Visvikis, D., et al. (2017). Tumor delineation and quantitative assessment of glucose metabolic rate within histologic subtypes of non–small cell lung cancer by using dynamic 18f fluorodeoxyglucose PET. *Radiology* 283, 547–559. doi: 10.1148/radiol.2016160329

Milne, E. N. (1967). Circulation of primary and metastatic pulmonary neoplasms: a postmortem microarteriographic study. *Am. J. Roentgenol.* 100, 603–619. doi: 10.2214/ajr.100.3.603

Milotti, E., Vyshemirsky, V., Sega, M., Stella, S., and Chignola, R. (2013). Metabolic scaling in solid tumours. *Sci. Rep.* 3, 1–6. doi: 10.1038/srep01938

Momcilovic, M., Jones, A., Bailey, S. T., Waldmann, C. M., Li, R., Lee, J. T., et al. (2019). *In vivo* imaging of mitochondrial membrane potential in non-small-cell lung cancer. *Nature* 575, 380–384. doi: 10.1038/s41586-019-1715-0

Montavon, G., Binder, A., Lapuschkin, S., Samek, W., and Müller, K.-R. (2019). "Layer-wise relevance propagation: an overview," in *Explainable AI: Interpreting, Explaining and Visualizing Deep Learning*, eds W. Samek, G. Montavon, A. Vedaldi, L. Hansen, and K. R. Müller (Springer), 193–209. doi: 10.10007/978-3-030-28954-6_10

Mori, S., Yamaji, K., Ishida, A., Prokushkin, S. G., Masyagina, O. V., Hagihara, A., et al. (2010). Mixed-power scaling of whole-plant respiration from seedlings to giant trees. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1447–1451. doi: 10.1073/pnas.0902554107

Murray, C. D. (1926). The physiological principle of minimum work: I. The vascular system and the cost of blood volume. *Proc. Natl. Acad. Sci. U.S.A.* 12:207. doi: 10.1073/pnas.12.3.207

Myatt, D., Hadlington, T., Ascoli, G., and Nasuto, S. (2012). Neuromantic – from semi-manual to semi-automatic reconstruction of neuron morphology. *Front. Neuroinform.* 6:4. doi: 10.3389/fninf.2012.00004

Newberry, M. G., Ennis, D. B., and Savage, V. M. (2015). Testing foundations of biological scaling theory using automated measurements of vascular networks. *PLoS Comput. Biol.* 11:e1004455. doi: 10.1371/journal.pcbi.1004455

Nguyen-Kim, T. D. L., Frauenfelder, T., Strobel, K., Veit-Haibach, P., and Huellner, M. W. (2015). Assessment of bronchial and pulmonary blood supply in non-small cell lung cancer subtypes using computed tomography perfusion. *Investig. Radiol.* 50, 179–186. doi: 10.1097/RLI.0000000000000124

Pandey, A. K., Singhi, E. K., Arroyo, J. P., Ikizler, T. A., Gould, E. R., Brown, J., et al. (2018). Mechanisms of VEGF (vascular endothelial growth factor) inhibitor–associated hypertension and vascular disease. *Hypertension* 71, e1–e8. doi: 10.1161/HYPERTENSIONAHA.117.10271

Pashayan, N., and Pharoah, P. D. P. (2020). The challenge of early detection in cancer. *Science* 368, 589–590. doi: 10.1126/science.aaz2078

Pawar, S., Dell, A. I., and Savage, V. M. (2012). Dimensionality of consumer search space drives trophic interaction strengths. *Nature* 486, 485–489. doi: 10.1038/nature11131

Pérez-García, V. M., Calvo, G. F., Bosque, J. J., León-Triana, O., Jiménez, J., Pérez-Beteta, J., et al. (2020). Universal scaling laws rule explosive growth in human cancers. *Nat. Phys.* 16, 1232–1237. doi: 10.1038/s41567-020-0978-6

Price, C. A., Weitz, J. S., Savage, V. M., Stegen, J., Clarke, A., Coomes, D. A., et al. (2012). Testing the metabolic theory of ecology. *Ecol. Lett.* 15, 1465–1474. doi: 10.1111/j.1461-0248.2012.01860.x

Prior, F. W., Clark, K., Commean, P., Freymann, J., Jaffe, C., Kirby, J., et al. (2013). "TCIA: an information resource to enable open science," in *Annual International Conference of the IEEE Engineering in Medicine and Biology Society, IEEE Engineering in Medicine and Biology Society* (NIH Public Access), 1282, 1282–1285. doi: 10.1109/embc.2013.6609742

Rao, S. R., Shelton, S. E., and Dayton, P. A. (2016). The "fingerprint" of cancer extends beyond solid tumor boundaries: assessment with a novel ultrasound imaging approach. *IEEE Trans. Biomed. Eng.* 63, 1082–1086. doi: 10.1109/TBME.2015.2479590

Ribeiro, F. L., dos Santos, R. V., and Mata, A. S. (2017). Fractal dimension and universality in avascular tumor growth. *Phys. Rev. E* 95:042406. doi: 10.1103/PhysRevE.95.042406

Richards, F. (1959). A flexible growth function for empirical use. *J. Exp. Bot.* 10, 290–301.

Salavati, A., Duan, F., Snyder, B. S., Wei, B., Houshmand, S., Khiewvan, B., et al. (2017). Optimal FDG PET/CT volumetric parameters for risk stratification in patients with locally advanced non-small cell lung cancer: results from the ACRIN 6668/RTOG 0235 trial. *Eur. J. Nuclear Med. Mol. Imaging* 44, 1969–1983. doi: 10.1007/s00259-017-3753-x

Samet, R., and Yildirim, Z. (2016). "A new methodology for blood vessel segmentation on lung CT images," in *2016 Nicograph International (NicoInt)* (IEEE), 1–7. doi: 10.1109/NicoInt.2016.1

Savage, V. M., Deeds, E. J., and Fontana, W. (2008). Sizing up allometric scaling theory. *PLoS Comput. Biol.* 4:e1000171. doi: 10.1371/journal.pcbi.1000171

Schmidt-Nielsen, K. (1984). *Scaling: Why Is Animal Size So Important?* Cambridge: Cambridge University Press.

Shojaii, R., Alirezaie, J., and Babyn, P. (2005). "Automatic lung segmentation in CT images using watershed transform," in *IEEE International Conference on Image Processing 2005* (IEEE), 1270. doi: 10.1109/ICIP.2005.1530294

Singadkar, G., Mahajan, A., Thakur, M., and Talbar, S. (2018). Automatic lung segmentation for the inclusion of juxtapleural nodules and pulmonary vessels using curvature based border correction. *J. King Saud Univ. Comput. Inform. Sci.* 1–13. doi: 10.1016/j.jksuci.2018.07.005

Soret, M., Bacharach, S. L., and Buvat, I. (2007). Partial-volume effect in PET tumor imaging. *J. Nuclear Med.* 48, 932–945. doi: 10.2967/jnumed.106.035774

Strahler, A. N. (1957). Quantitative analysis of watershed geomorphology. *EOS Trans. Am. Geophys. Union* 38, 913–920.

Sullivan, D. C., Obuchowski, N. A., Kessler, L. G., Raunig, D. L., Gatsonis, C., Huang, E. P., et al. (2015). Metrology standards for quantitative imaging biomarkers. *Radiology* 277, 813–825. doi: 10.1148/radiol.2015142202

Tekin, E., Hunt, D., Newberry, M. G., and Savage, V. M. (2016). Do vascular networks branch optimally or randomly across spatial scales? *PLoS Comput. Biol.* 12:e1005223. doi: 10.1371/journal.pcbi.1005223

Turcotte, D., Pelletier, J., and Newman, W. (1998). Networks with side branching in biology. *J. Theoret. Biol.* 193, 577–592.

Valk, P. E., Delbeke, D., Bailey, D. L., Townsend, D. W., and Maisey, M. N. (2006). *Positron Emission Tomography: Clinical Practice*. London: Springer Science & Business Media.

Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009). Understanding the warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033. doi: 10.1126/science.1160809

Visser, E. P., Philippens, M. E., Kienhorst, L., Kaanders, J. H., Corstens, F. H., de Geus-Oei, L.-F., et al. (2008). Comparison of tumor volumes derived from glucose metabolic rate maps and SUV maps in dynamic 18F-FDG PET. *J. Nuclear Med.* 49, 892–898. doi: 10.2967/jnumed.107.049585

Vogl, T. J., Shafinaderi, M., Zangos, S., Lindemayr, S., and Vatankhah, K. (2013). "Regional chemotherapy of the lung: transpulmonary chemoembolization in malignant lung tumors," in *Seminars in Interventional Radiology* (Thieme Medical Publishers), 30, 176–184. doi: 10.1055/s-0033-1342959

Von Bertalanffy, L. (1957). Quantitative laws in metabolism and growth. *Q. Rev. Biol.* 32, 217–231.

Wang, X., Leader, J. K., Wang, R., Wilson, D., Herman, J., Yuan, J.-M., et al. (2017). Vasculature surrounding a nodule: a novel lung cancer biomarker. *Lung Cancer* 114, 38–43. doi: 10.1016/j.lungcan.2017.10.008

Warburg, O. (1956). On the origin of cancer cells. *Science* 123, 309–314.

West, G. B., Brown, J. H., and Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science* 276, 122–126.

West, G. B., Brown, J. H., and Enquist, B. J. (1999). A general model for the structure and allometry of plant vascular systems. *Nature* 400, 664–667.

West, G. B., Brown, J. H., and Enquist, B. J. (2001). A general model for ontogenetic growth. *Nature* 413:628. doi: 10.1038/35098076

West, G. B., Enquist, B. J., and Brown, J. H. (2009). A general quantitative theory of forest structure and dynamics. *Proc. Natl. Acad. Sci. U.S.A.* 106, 7040–7045. doi: 10.1073/pnas.0812294106

Wright, S. N., Kochunov, P., Mut, F., Bergamino, M., Brown, K. M., Mazziotta, J. C., et al. (2013). Digital reconstruction and morphometric analysis of human

brain arterial vasculature from magnetic resonance angiography. *Neuroimage* 82, 170–181. doi: 10.1016/j.neuroimage.2013.05.089

Wu, C., Hormuth, D. A., Oliver, T. A., Pineda, F., Lorenzo, G., Karczmar, G. S., et al. (2020). Patient-specific characterization of breast cancer hemodynamics using image-guided computational fluid dynamics. *IEEE Trans. Med. Imaging* 39, 2760–2771. doi: 10.1109/TMI.2020.2975375

Yao, Y., Jumabay, M., Wang, A., and Boström, K. I. (2011). Matrix gla protein deficiency causes arteriovenous malformations in mice. *J. Clin. Investig.* 121:2993. doi: 10.1172/JCI57567

Yekutieli, I., and Mandelbrot, B. (1994). Horton-strahler ordering of random binary trees. *J. Phys. A Math. Gen.* 27:285.

Yuan, X., Zhang, J., Ao, G., Quan, C., Tian, Y., and Li, H. (2012). Lung cancer perfusion: can we measure pulmonary and bronchial circulation simultaneously? *Eur. Radiol.* 22, 1665–1671. doi: 10.1007/s00330-012-2414-5

Zamir, M. (2006). *The Physics of Coronary Blood Flow*. New York, NY: Springer Science & Business Media.

Zhou, M., Leung, A., Echegaray, S., Gentles, A., Shrager, J. B., Jensen, K. C., et al. (2018). Non-small cell lung cancer radiogenomics map identifies relationships between molecular and imaging phenotypes with prognostic implications. *Radiology* 286, 307–315. doi: 10.1148/radiol.2017161845

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frontiers in Ecology and Evolution

HYPOTHESIS AND THEORY
published: 08 October 2021
doi: 10.3389/fevo.2021.741389

# Using Free-Range Laboratory Mice to Explore Foraging, Lifestyle, and Diet Issues in Cancer

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As cancer progresses, its impact should manifest in the foraging behavior of its host much like the effects of endo-parasites that hinder foraging aptitudes and risk management abilities. Furthermore, the lifestyle of the host can impact tumor growth and quality of life. To approach these questions, we conducted novel experiments by letting C57BL/6 laboratory mice, with or without oral squamous cell carcinoma, free range in a large outdoor vivarium. Our goals were to: (1) determine whether one could conduct experiments with a mouse model under free range conditions, (2) measure effects of cancer burden on foraging metrics, (3) compare tumor growth rates with laboratory housed mice, and (4) begin to sort out confounding factors such as diet. With or without cancer, the C57BL/6 laboratory mice dealt with natural climatic conditions and illumination, found shelter or dug burrows, sought out food from experimental food patches, and responded to risk factors associated with microhabitat by foraging more thoroughly in food patches under bush (safe) than in the open (risky). We quantified foraging using giving-up densities of food left behind in the food patches. The mice's patch use changed over time, and was affected by disease status, sex, and microhabitat. Males, which were larger, consumed more food and had lower giving-up densities than females. Relative to cancer-free mice, mice with growing tumors lost weight, harvested more food, and increasingly relied on patches in the bush microhabitat. The tumors of free-ranging mice in the vivarium grew slower than those of their cohort that were housed in mouse cages in animal facilities. Numerous interesting factors could explain the difference in tumor growth rates: activity levels, stress, weather, food intake, diet, and more. To tease apart one of these intertwined factors, we found that tumors grew faster when mice in the laboratory were fed on millet rather than laboratory mouse chow. While just a start, these novel experiments and framework show how free-ranging mice provide a model that can test a broader range of hypotheses and use a broader range of metrics regarding cancer progression and its consequences for the host.

**Keywords: disease ecology, foraging ecology, foraging aptitudes, risk management, cancer, tradeoffs of food and safety, tumor growth rates, environmental effects**

OPEN ACCESS

***Edited by:***
*Frederick R. Adler, The University of Utah, United States*

***Reviewed by:***
*James Ruff, The University of Utah, United States*
*Wendy Hood, Auburn University, United States*
*KayLene Yamada, Auburn University, United States, in collaboration with reviewer WH*

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***Specialty section:***
*This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *14 July 2021*
***Accepted:*** *06 September 2021*
***Published:*** *08 October 2021*

***Citation:***
*Makin DF, Agra E, Prasad M, Brown JS, Elkabets M, Menezes JFS, Sargunaraj F and Kotler BP (2021) Using Free-Range Laboratory Mice to Explore Foraging, Lifestyle, and Diet Issues in Cancer. Front. Ecol. Evol. 9:741389. doi: 10.3389/fevo.2021.741389*

## INTRODUCTION

Cancer experiments with mice invariably involve very small spaces (laboratory cages), *ad lib* food, the simplest of lifestyles, and little to no habitat heterogeneity. Here we explore the potential for using mouse model experiments in large outdoor enclosures or vivaria using the inbred laboratory mouse strain C57BL/6. This strain dates from 1921 and may be the most widely used mouse model in research (Festing, 1979; Rao et al., 1988; Song and Hwang, 2017). We offered each of four groups of C57BL/6 laboratory mice (male vs. female; and cancer vs. cancer-free) an outdoor space measuring 8.5 m × 17 m. The results are promising and instructive with respect to insights into foraging behaviors, tumor growth rates, and diet.

Experiments in large outdoor enclosures have a long history in ecological studies. Enclosures have ranged in size from a few square meters in an indoor setting (e.g., Dice, 1945; Clarke, 1983; Morris et al., 2017; Mowry et al., 2017), somewhat larger semi-natural outdoor enclosures of one to several hundred square meters (e.g., Brown et al., 1988; Kotler et al., 1991; Meagher et al., 2000; Bär et al., 2020), on up to large natural outdoor enclosures of one to many hectares (e.g., Abramsky et al., 1991, 1997, Rohner and Krebs, 1996). The subject matters of these studies are diverse and range from ecological questions such as food-safety trade-offs, locomotion, and territoriality (Morris et al., 2017) to evolutionary issues such as body mass and sexual selection (Meagher et al., 2000).

More recently, semi-natural enclosures have been used to study both wild-caught and laboratory-bred house mice (e.g., Ruff et al., 2017; Phifer-Rixey et al., 2018), including various "rewilding" experiments with C57BL/6 laboratory mice (Leung et al., 2018; Cope et al., 2019; Bär et al., 2020; Graham, 2021). Medically related questions with laboratory mice have included the role of fungi in immune function (Yeung et al., 2020), the effects of high levels of sugar consumption on mortality (Ruff et al., 2013), the role of sex and social organization on rates of pathogen transmission (Cornwall et al., 2021), and the magnitude of inbreeding effects (Meagher et al., 2000). Such settings can measure the performance of mice as an assay for the safety of pharmaceuticals (Gaukler et al., 2016a,b).

Large outdoor vivaria have shown particular utility in foraging ecology (e.g., Brown et al., 1988; Kotler et al., 1991, 2010; Embar et al., 2011). In such experiments, rodents respond to predators (e.g., owls, snakes, and foxes) by foraging less and shifting foraging toward safer microhabitats. The vivarium used in the current study also provided the setting for experiments with wild gerbils and other desert rodents to address issues ranging from the interplay of state, time allocation, and vigilance in optimal foraging decisions over the lunar cycle (Kotler et al., 2010), the role of sight lines (Embar et al., 2011), and the consequences of compromise-breaking adaptations in understanding limited convergence between rodents from different continents (Kotler et al., 2016). With respect to disease burden, wild-caught gerbils infected with an endoparasitic bacteria (*Mycoplasma haemomuris*-like bacteria) harvested less food, exhibited less effective anti-predator responses, and were more susceptible to predation by owls (Makin et al., 2020). Cancer burdens in mouse models might elicit similar responses. Free-range experiments in large enclosures represent a scaling down of space when using wild-caught rodents, whereas they represent a scaling up for laboratory mice that may not have seen a space larger than a laboratory cage in 100 or more generations.

In the vivarium, as in many field studies of rodents, foraging behaviors can be measured using experimental food patches in which a known amount of seeds is mixed into a substrate that requires the rodent to dig, search for, and harvest seeds. This creates diminishing returns: as the patch becomes depleted, the animal's harvest rate declines. Eventually, the forager abandons the patch. The seeds remaining in the patch, the giving-up density (GUD), can be collected and measured to provide data on the amount of seeds harvested and the willingness of the forager to work for additional food. The less food left, the more the animal was willing to work. The giving-up density declines (more food harvested) in patches perceived as safer from predation risk, and for animals that have a higher need for food (Brown, 1988, 1992). Giving-up densities in such food patches can provide both behavioral enrichments and measures of foraging behavior in the free-ranging C57BL/6 mice.

C57BL/6 mice free-ranging in the vivarium need to contend with natural fluctuations in temperature and the need to thermoregulate. There, they are no longer housed under sterile or near-sterile conditions. They must spend several hours each night visiting seed trays and digging through the sand to find and harvest seeds. The mice in the vivarium must seek shelter and avoid risky locations. In general, studies reveal that wild-caught rodents perceive greater predation risk away from cover vs. under cover (open vs. bush microhabitat) (Brown et al., 1988; Kotler et al., 1991), have lower giving-up densities in the bush microhabitat (Brown et al., 1988; Kotler et al., 1991; Kotler and Blaustein, 1995), and have lower giving-up densities when in a lower body condition or when experiencing a greater need for food (Kotler, 1997; Kotler et al., 2004; Berger-Tal and Kotler, 2010; Berger-Tal et al., 2010). Under this more complex lifestyle, we can test hypotheses for how cancer and its progression influence food harvest, giving-up densities, and use of open and bush microhabitats.

Exercise (Zielinski et al., 2004; Jones et al., 2012; Betof et al., 2015; Idorn and Thor Straten, 2017), food limitation (Lee et al., 2012; Nencioni et al., 2018), and stress (Grimm et al., 1996; Thaker et al., 2006; Kokolus et al., 2013) all have been shown to affect tumor growth rates, generally resulting in slower tumor growth rates. Free-ranging C57BL/6 mice as compared to their counterparts remaining in the laboratory will likely exercise more, contend with food limitation, and experience more and different forms of stress. To explore this, we compared tumor growth rates of a cohort of mice that were inoculated with the same batch of cancer cells on the same day, and then split into those remaining in the laboratory and those transferred to the vivarium.

Diet is known to influence tumor growth rates (e.g., Wang et al., 1995; Rose et al., 1999; O'Neill et al., 2016). To measure patch use and for consistency with other studies with wild-caught rodents, we used millet seeds. To initiate this novel and unusual experiment, the animal care committee allowed us to use millet

for the free-range mice, but not for those housed in the laboratory (thus confounding free-range with diet regarding tumor growth rates). With the successful application of this approach to the free-range mice, we subsequently received permission to compare tumor growth rates of laboratory mice fed on mouse chow pellets or fed on just millet. Millet is lower in protein than the mouse chow pellets, and high protein diets have been shown to slow tumor growth in mouse models (Ho et al., 2011) and associated ketogenic diets (Chung and Park, 2017; Weber et al., 2020). Alternatively, millet is known to be high in antioxidants, often seen as anti-tumorigenic (Parohan et al., 2019). In addition to these alternative hypotheses, millet provides a compelling food source. Millet is one of the ancient grains associated with the Fertile Crescent. It is likely associated with the original evolution and dispersal of the ancestor of C57BL/6 laboratory mice, the house mouse, *Mus musculus domesticus* (Cucchi and Vigne, 2006; Cucchi et al., 2020), as well as the house sparrow, *Passer domesticus* (Boursot et al., 1993; Whelan et al., 2015). These animals and their laboratory descendants may be specialists at consuming cereal grains such as millet.

## METHODS

For all experiments, we used C57BL/6 laboratory mice. For cancer research, they are favored for being permissive of many injected cancer cell lines while remaining immuno-competent.

### Effects of Microhabitat, Sex, and Cancer on Foraging Behaviors

C57BL/6 mice are being used more frequently within the free-range contexts of outdoor enclosures to address ecological, evolutionary, and even medical questions (e.g., Gaukler et al., 2016a,b; Cope et al., 2019; Bär et al., 2020; Yeung et al., 2020). With Experiment 1A, we tested whether free ranging, C57BL/6 laboratory mice forage in a manner typical of house mice and other seed-eating rodents in nature; and we tested for the effects of cancer on foraging behavior.

Experiment 1A took place in a large, outdoor vivarium (**Figure 1**) on the Sede Boker Campus of Ben-Gurion University of the Negev in Midreshet Ben-Gurion, Israel (30.857274°N, 34.780942°E) and Experiment 1B in laboratories located there and on the Marcus Family Campus of Ben-Gurion University in Beer Sheva, Israel. Sede Boker is a small rural town of less than 1,000 inhabitants located on the 90 mm rainfall isopleth in the Negev Desert. The vivarium is located there on the desert's edge. The vivarium is exposed to ambient conditions, including illumination, humidity, and temperature. Natural predators in the vicinity include barn owls (*Tyto* alba), red foxes (*Vulpes vulpes*), and Clifford's desert diadem snakes (*Spalerosophis cliffordi*). During the vivarium experiments, daytime highs averaged 33.56 ± 0.244 (s.e.) °C and nighttime lows 19.59 ± 0.463 (s.e.) °C. No precipitation fell then, but dew occurred on most nights. The vivarium is a rectangular outdoor enclosure (17 × 34 × 4.5 m high) enclosed with chicken wire sides and roof. It is also bounded with a rodent-proof wall that extends 1 m below ground and another 1 m above ground.

**FIGURE 1 |** The vivarium.

Inside the vivarium, two 1 m tall and 1 m deep walls run east to west and north to south, respectively. These walls divide the vivarium into four equally sized 17 × 8.5 m quadrants. We placed 5 water dishes and three nest boxes in each quadrant. Mice were free to occupy nest boxes or burrows that they could either find or dig for themselves. We provided seed resources to rodents using experimental food patches, and quantified rodent foraging by measuring GUDs (giving-up densities: the amount of food left behind in a resource patch after an animal has stopped feeding from the patch) in these patches. Except for the seeds that we provided in the food patches, there was little other food to be found.

Food patches consisted of plastic trays (25 × 25 × 10 cm) each filled with 3 l of sifted sand. Before each night of the experiment, we provisioned each tray with 6 g of millet seeds (11% crude protein, 4% crude fat, 8.5% crude fiber, total carbohydrates 73%) mixed thoroughly and randomly into the sand. Each quadrant received four pairs of trays for a total of 32 patches in the vivarium. Each pair of trays was separated from the adjacent pair by approximately 2–3.5 m. The trays within each pair were placed 1.5 m apart. One tray of each pair was covered by a low-lying wooden trellis (76 × 60 × 16 cm) covered in black shade cloth. This tray simulated the bush microhabitat typical of natural systems. The other tray of the pair was left uncovered and simulated the open microhabitat.

Most rodent species tested to date, whether in enclosures or free-living in nature, find the bush microhabitat to be safer than the open (e.g., Brown et al., 1988; Kotler et al., 1991;

Longland and Price, 1991). They demonstrate this preference by harvesting more seeds from and having lower GUDs in trays placed in the bush microhabitat relative to those placed in the open microhabitat (e.g., Kotler et al., 1991, but see Brown, 1989 for a reversal of this pattern in the kangaroo rat, *Dipodomys merriami*). Prior researchers have shown that both laboratory rats and laboratory mice have lower GUDs when patches are covered (Arcis and Desor, 2003; Troxell-Smith et al., 2016). *We expected laboratory mice in the vivarium to exhibit lower GUDs in the bush than in the open.*

Prior to the vivarium experiment, 10 mice each marked with a uniquely numbered PIT (passive induction transponder) tag were placed in each quadrant (40 in total) of the vivarium. In two of the quadrants, all the mice were males; in two of the quadrants, all were females. This allowed us to test for sex differences in GUDs. *The sex that finds energy more valuable or that feels safer will have the lower GUD.*

To begin the experiment, all mice in two of the quadrants (one with 10 males; one with 10 females) were injected subcutaneously with a mouse-derived cancer cell line. The 20 mice in the remaining two quadrants were injected with saline. The cell line is derived from 4-Nitroquinoline N-oxide (4NQO) induced oral squamous cell carcinoma from a male C57BL/6 mouse (Hawkins et al., 1994; Badarni et al., 2019). We have sequenced and characterized these cell lines (Elkabets and Prasad, unpublished data). Prior to injection in the mice, the cell line was grown and maintained at 37°C in a humidified atmosphere at 5% $CO_2$ in DMEM media supplemented with 1% l-glutamine 200 mM, 100 units each of penicillin and streptomycin, and 10% FBS. Cells were routinely tested for *Mycoplasma* infection and treated with appropriate antibiotics as needed (De-Plasma, TOKU-E, D022). For injection, cells were trypsinized and resuspended in 1X sterile PBS (c. 1 Million cells /injection). We made two subcutaneous injections per mouse, one in the left and one in the right flank (100 μl for each injection). In this way, each mouse developed two localized non-metastatic tumors. The control group was injected with sterile 1xPBS (100 μl in each side). All mice in both the experimental group and the control group were anesthetized for the procedures. Anesthesia was performed using an intraperitoneal injection of ketamine (80–100 mg/kg) and xylazine (10–12.5 mg/kg), following which mice were placed on a heating pad for recovery. *Relative to the cancer-free mice, the cancer mice over time should see an increase in GUDs if their capacity to forage becomes impaired, or they may show a decrease in GUDs if they require more seeds to meet metabolic demands.*

We quantified tumor volume twice a week by measuring length (mm) and width (mm) using a caliper and fitting them to the formula Volume ($mm^3$) = ($Length^2 \times Width \times \pi$)/6. At the conclusion of the experiment, we also weighed final tumor mass. All animals were weighed weekly. For measurements, we either captured animals by hand from nest boxes or used Sherman live traps baited with millet seeds on nights without food patches. We injected all mice of Experiment 1 with cancer cells or with saline on 30 July 2020. Following this, we released mice into the vivarium a day after injection.

For mice in the vivarium, we gave them 2 days after release to acclimate, after which we began collecting GUD data from the seed trays on 3 August 2020. Our experience with other species of rodents indicates that 2 days is sufficient acclimation to yield reliable data. Each week, for the next 4 weeks, we collected GUD data on four consecutive days centered on the moon phase and starting with the full moon. Prior to each night of data collection, we provisioned each seed tray with millet seeds (6 g). The following morning, we collected the remaining seeds from each tray and replenished seeds and sand to their original levels. We then returned the seeds to the laboratory where they were dried, cleaned of sand and debris, and weighed to obtain the GUD for each tray.

We analyzed the GUD data using an ANCOVA, with day as a covariate and GUD in a patch as the dependent variable (in grams). The rationale is that the tumors are growing with time and should change the disposition of the mice with cancer relative to those without. This will manifest as a day by cancer treatment interaction. Changes in risk management with time will manifest as a day by cancer treatment by microhabitat interaction. Hence, our interest is primarily in the interactions of group variables with day. The group variables were cancer treatment (cancer and cancer-free), sex (male and female), and microhabitat (bush and open). We included in our model the covariate of day and main effects of sex and microhabitat; the interaction of sex by microhabitat; the interaction of treatment by day; the interaction of treatment by sex by day; the interaction of treatment by microhabitat by day; the interaction of treatment by sex by microhabitat by day. Interactions of group variables (and their interactions) with day indicate differences in slopes, or rates at which GUDs changed per day. The data collection included the entire lunar cycle, but we did not include moon phase in our analysis as moon phases are associated with day, which in turn are associated with tumor growth in the cancer mice. As such, the effect of moon phase is subsumed in the covariate of day.

## Effect of Free-Ranging on Tumor Growth Rates

Experiment 1B involved measuring the tumor growth rates of mice housed under standard laboratory conditions. Drawing randomly from the same set of mice as Experiment 1A and using the same batch of cancer cells on the same day (30 July 2020), we injected mice that were then kept in two laboratory locations: Sede Boker (3 males and 3 females) and Beer Sheva (5 males and 6 females). The Beer Sheva facility maintains SPF (Specific Pathogen Free) conditions, the Sede Boker facility does not. Because of the animal care regulations for the SPF conditions at the Beer Sheva facilities, mice in Beer Sheva could only be fed sterilized mouse chow pellets. Accordingly, mice in both the Sede Boker and Beer Sheva laboratories received mouse chow pellets (Ssnif, Mouse Breeding V1154-300, 22.5% crude protein, 5.5% crude fat, 4.0% crude fiber, 6.0% crude ash, 1.0% calcium, 0.7% calcium). As in Experiment 1A, we quantified tumor volume twice a week, weighed animals weekly, and, at the conclusion of the experiment, weighed final tumor masses.

We analyzed the tumor growth rates in a similar manner as the GUD data. We summed the tumor volume from both sides of a mouse and then under the assumption of exponential

tumor growth, we took the natural logarithm of this value as our dependent variable. For the ANCOVA of the tumor growth rates measured during Experiment 1 (the vivarium and the concurrent laboratory trials), day was the covariate, with location (free-range in the vivarium, Beer Sheva lab, and Sede Boker lab) and sex (male and female) as group variables.

## Effect of Diet on Tumor Growth Rates

In comparing tumor growth rates between mice of Experiments 1A and 1B there is a confounding of diet and location. To test for effects of a diet of millet or mouse chow pellets on tumor growth rates, we ran Experiment 2 in the laboratory in Sede Boker where both millet and mouse chow pellets were permitted. We began by giving each individual in two groups of mice cancer as described above. One group then received millet *ad lib,* the other mouse chow *ad lib*. Each group consisted of 6 males and 6 females for a total of 24 mice. We injected mice with cancer cells as described above on November 30, 2020. We weighed mice weekly and measured tumor volume twice a week. We ended the experiment on January 4, 2021, euthanized individual mice, and then resected, weighed, and preserved tumors.

For tumor growth rates in response to diet, we used the same ANCOVA as with tumor growth in the three locations, but with the adjustment that diet (millet and mouse chow pellets) substituted for location as a group variable. While seemingly appealing, we did not analyze tumor growth rates from both experiments together, as each of the two experiments used a different batch of cultured cancer cells, and batch effects can be significant (e.g., Karp et al., 2020). The same batch was used for all mice within an experiment.

For all experiments, we also used ANCOVA (one for each experiment) to track changes in body mass and the effects of the group variables, including housing conditions, cancer vs. cancer-free, and diet, as appropriate.

# RESULTS

## Effects of Microhabitat, Sex, and Cancer on Foraging Behaviors

The ANCOVA of the GUDs in Experiment 1A provided a good fit to the data ($r^2 = 0.73$). As expected, mouse GUDs were significantly and substantially higher in the open [4.23 ± 0.08 (s.e.) g] than bush [1.72 ± 0.06 (s.e.) g] microhabitat [MS = 802.1, $F_{(1,\ 496)} = 1075.2$, $p < 0.001$]. Furthermore, males had significantly lower GUDs than females: 2.48 ± 0.08 (s.e.) g vs. 3.48 ± 0.11 (s.e.) g [MS = 135.3, $F_{(1,\ 496)} = 181.4$, $p < 0.001$]. Note that males are larger in body mass than females. Males might also be more territorial than females (more often found alone in nest boxes rather than in groups), although we did not quantify this. Overall, males harvested 5.63 g of millet per day per individual, and females harvested 4.63 g per day per individual. Rather than consuming all of this, the mice can be expected to have cached some. Most notably, there is a significant interaction effect between microhabitat and sex (**Figure 2**) showing that the higher GUDs of females relative to males was much more pronounced in the open than bush microhabitat [MS = 48.5, $F_{(1,\ 496)} = 65.0$, $p < 0.001$]. This result suggests that females are warier of the risky open areas than males (see Kotler et al., 1988, 1991 for experimental evidence showing that differences in GUDs across microhabitat is caused by predation risk).

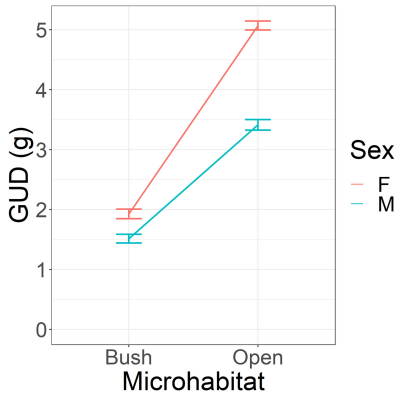


**FIGURE 2 |** Giving-up densities (GUD) of mice in the vivarium according to microhabitat (bush and open) and sex. Females (F) have lower GUDs than males (M). All mice had lower GUDs in the bush than the open. Error bars represent standard errors of the means.

There were significant temporal trends in GUDs during the experiment, during which time the tumors were growing in the mice with cancer. Overall, GUDs tended to decline with time at a rate of −0.017 g per day [MS = 10.28, $F_{(1,\ 496)} = 13.78$, $p < 0.001$]. There was a significant microhabitat by cancer treatment by day effect [MS = 4.12, $F_{(1,\ 496)} = 5.53$, $p < 0.02$], showing that a growing tumor burden influenced foraging behavior. In the bush microhabitat, the GUDs of cancer mice changed little with time (overall rate of increase of 0.001 g per day) while that of cancer-free mice increased substantially with time (0.035 g per day). In the open microhabitat, the GUDs of both cancer and cancer-free mice declined similarly with time (−0.050 and −0.057 g per day, respectively), with cancer mice tending to have higher GUDs than cancer-free mice. While all mice began to shift more of their foraging toward the open microhabitat with time (suggesting perhaps a growing sense of safety), this trend was stronger for the cancer-free mice than for the cancer mice. This suggests possibly greater divergence in wariness over the course of the experiment by the cancer mice as their tumor burdens increased.

There was a significant cancer treatment by sex by day effect on GUDs [MS = 5.17, $F_{(1,\ 496)} = 6.925$, $p < 0.01$; **Figures 3A,B**). For females, cancer and cancer-free mice saw a decline in GUDs with time, though more strikingly for females with cancer than those without (−0.034 and −0.013 g per day, respectively). For males, declines in GUDs with time were less pronounced than for females, with less difference between cancer and cancer-free males (−0.016 and −0.009 g per day, respectively). There was a significant cancer treatment by sex by microhabitat by day effect [MS = 4.09, $F_{(1,\ 496)} = 5.485$, $p < 0.02$]. It shows that in the open microhabitat all four groups of mice (cancer treatment by sex) exhibited nearly identical daily declines in GUDs (**Figure 3B**), with GUDs for cancer mice being higher than cancer-free mice.

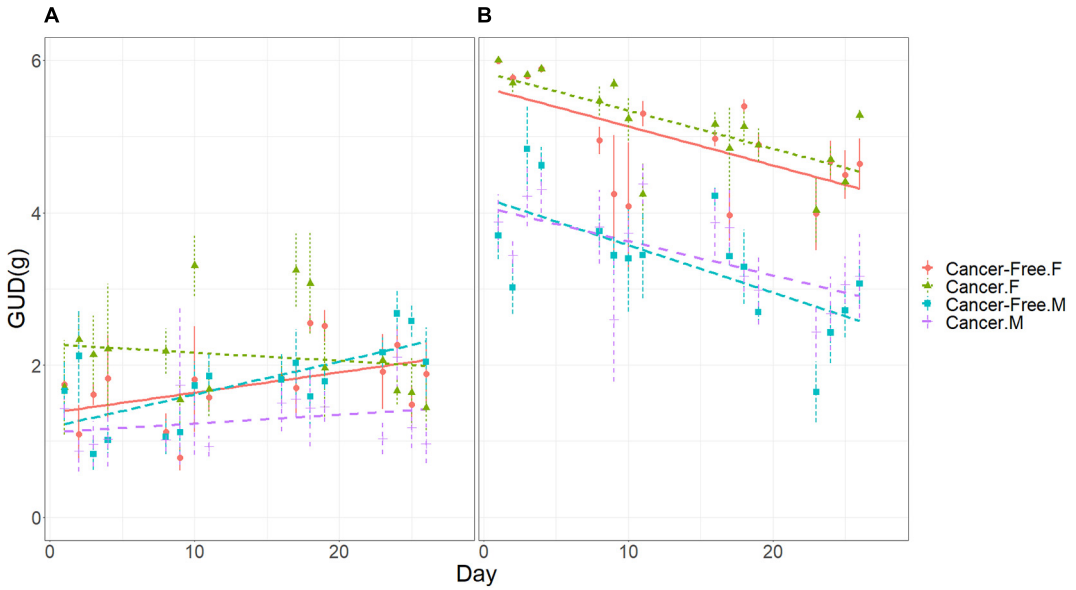


**FIGURE 3 |** Giving-up densities (GUDs) of mice in the vivarium according to cancer treatment, sex, and date. **(A)** Bush microhabitat. **(B)** Open microhabitat. Error bars represent standard errors of the means.

This is suggestive of higher foraging costs for cancer mice. But in the bush microhabitat, there were striking divergences in daily trends, with cancer-free males and females showing sharp increases in GUDs with time, cancer males exhibiting a smaller increase in GUDs with time, and cancer females showing a temporal decline in daily GUDs (**Figure 3A**). By the end of the experiment, cancer males achieved lower GUDs in the bush than cancer-free males. This suggests increasing energy demands for cancer mice relative to cancer free mice.

## Effect of Free-Ranging on Tumor Growth Rates

In Experiment 1 (A and B), both sex and housing conditions (free-range, Sede Boker facility, and Beer Sheva facility) influenced tumor growth rates. Mice at all locations started the experiment with similar tumor volumes (**Figure 4B**). Furthermore, the initial tumor volume was larger for males than females and remained so throughout the experiment [**Figure 4A**; M.S. = 9.483, $F_{(1,\ 256)} = 55.6$, $p < 0.001$]. Tumor volume increased at a rate of 9.4% per day during the experiment [M.S. = 127.7, $F_{(1,\ 256)} = 748.7$, $p < 0.001$], with similar rates for males and females. Finally, tumor growth rates differed in the three locations, with similarly high rates in the laboratory of 10.5% per day in Beer Sheva and 9.7% per day in Sede Boker, and a much lower rate of 8.0% per day in the free-range vivarium mice [**Figure 4B**; M.S. = 1.165, $F_{(1,\ 256)} = 6.83$, $p = 0.001$]. The growth rate differences between caged facilities and the free-range vivarium matters. Exponential growth rates of 10% vs. 8% per day will result in a tumor that is 80% larger after 30 days (20-fold vs. 11-fold increase).

In terms of body mass, males were significantly larger than females in Experiment 1 (A and B) (23.7 vs. 19.0 g, respectively, at day zero). For free-ranging mice in the vivarium, those with cancer declined in body mass over time (−0.024 grams per day) while those without saw little or no change (−0.006 g per day) [ANCOVA: significant interaction of cancer treatment with day, M.S. = 30.89, $F_{(1,\ 198)} = 6.73$, $p < 0.01$)] (**Figure 5A**). This occurred despite the mice with cancer increasing their daily food harvest relative to the cancer-free mice over time. In a separate analysis, for mice with cancer, those in the laboratory increased in body mass over the 31 days of the experiment (0.06 g per day), while as noted, those in the vivarium declined in mass with time [ANCOVA: significant interaction of location by day, M.S. = 30.89, $F_{(1,\ 198)} = 6.73$, $p < 0.01$] (**Figure 5B**). Although males are heavier than females, the location specific trends were similar for both sexes (**Figure 5A**).

During Experiment 1A, one cancer mouse male was found dead in the vivarium on 17 August and another cancer mouse male on 20 August. Each was replaced the following day by a cancer mouse male kept in the laboratory for such purposes. In addition, a cancer mouse female and a cancer-free female were killed on 13 August by a snake that managed to enter the vivarium. The snake was removed and released, and the mice were replaced on August 14th.

## Effect of Diet on Tumor Growth Rates

In Experiment 2 conducted in the Sede Boker facility, diet significantly affected tumor growth rates. As before, the initial tumor volume was larger for males than females [M.S. = 0.932, $F_{(1,210)} = 6.058$, $p < 0.02$], and thereafter remained larger as tumors in males and females grew at the same rate (**Figure 6A**). Tumors grew at a rate of 9.3% per day [M.S. = 156.14, $F_{(1,210)} = 1015.7$, $p < 0.001$]. Diet did not affect initial mass, but did influence tumor growth rates. Mice fed on millet had

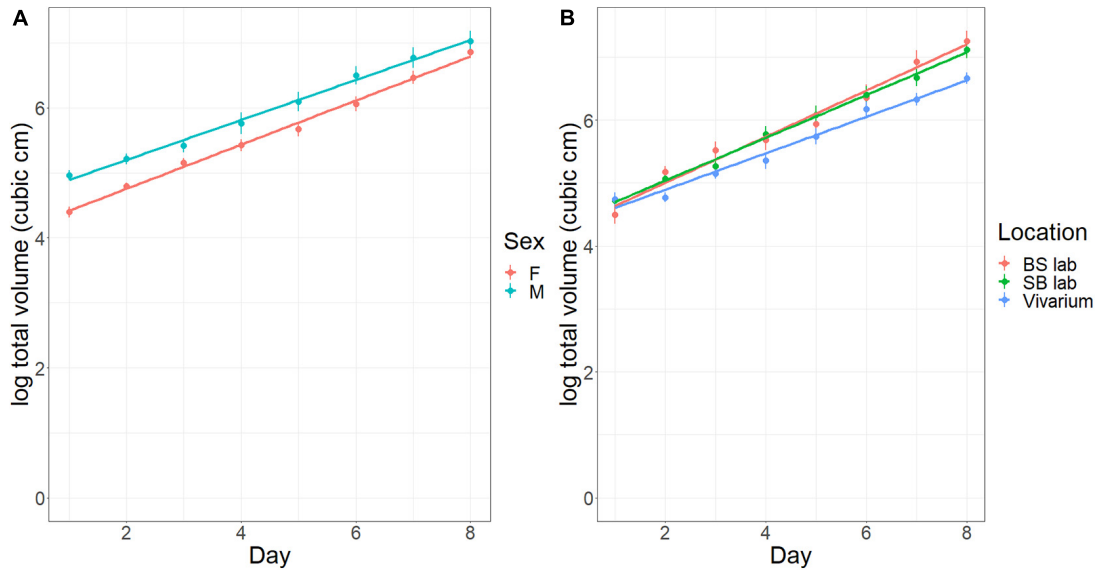


**FIGURE 4 |** Tumor size ($cm^3$) in the vivarium experiment and concurrent laboratory experiment over time (days). **(A)** Effect of sex (F, female; M, male). **(B)** Effect of Location (BS laboratory, laboratory in Beer Sheva with SPF conditions; SB laboratory, laboratory in Sede Boker; Vivarium, large, outdoor vivarium in Sede Boker). Error bars represent standard errors of the means.

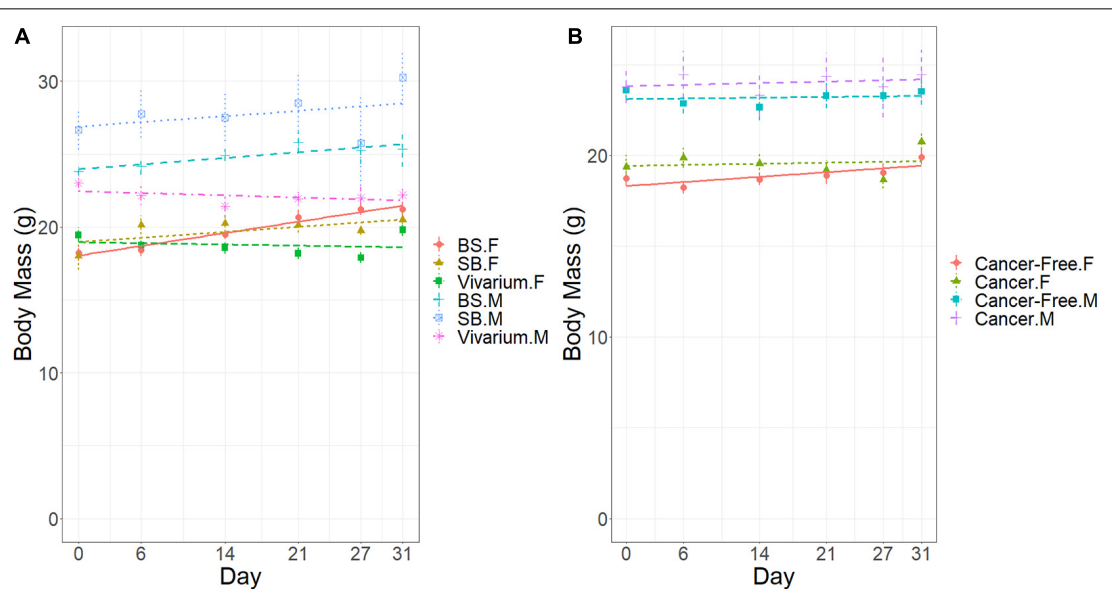


**FIGURE 5 |** Body mass (g) of the mice in the vivarium experiment over time (days). **(A)** Effects of sex (F, female; M, male) and location (Vivarium, Laboratory). **(B)** Effects of sex (F, female; M, male) and cancer treatment (Yes, cancer; cancer-free). Error bars represent standard errors of the means.

significantly higher tumor growth rates than mice eating mouse chow pellets [M.S. = 1.385, $F_{(1,\ 210)} = 9.007$, $p = 0.003$] at 9.8% per day and 8.8% per day, respectively (**Figure 6B**). After 30 days, this difference leads to tumors that are 35% larger in mice fed on millet than those fed on mouse chow.

In terms of body mass in Experiment 2, like the first experiment, males were larger than females [23.7 ± 0.4 (s.e.) g vs. 18.9 ± 0.4 (s.e.) g, respectively, at day zero], and body mass increased over time for both diet treatments (0.089 g per day), with a strong trend of mice fed on millet gaining mass at a faster rate than those fed on mouse chow pellets (0.122 g per day vs. 0.055 g per day, respectively) [ANCOVA: interaction of diet with day, M.S. = 11.67, $F_{(1,\ 140)} = 3.45$, $p = 0.065$] (**Figure 7**).

For both Experiments, the final mass of a tumor correlated tightly with the final measurement of tumor volume [Pearson's correlation of 0.932 and 0.882 for the first ($n = 67$) and second ($n = 48$) experiments, respectively]. Recall that each cancer mouse had two tumors, one on its left flank and one on its right.

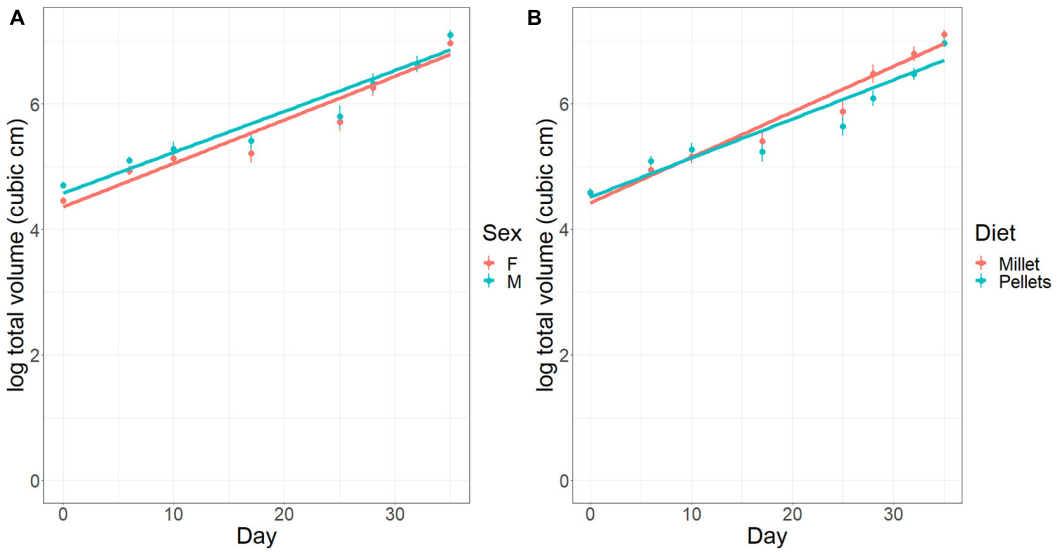


**FIGURE 6 |** Tumor size ($cm^3$) in the laboratory diet experiment over time (days). **(A)** Effect of sex (F, female; M, male). **(B)** Effect of diet (Millet, mice fed on millet seeds; Pellets, mice fed on mouse chow pellets). Error bars represent standard errors of the means.

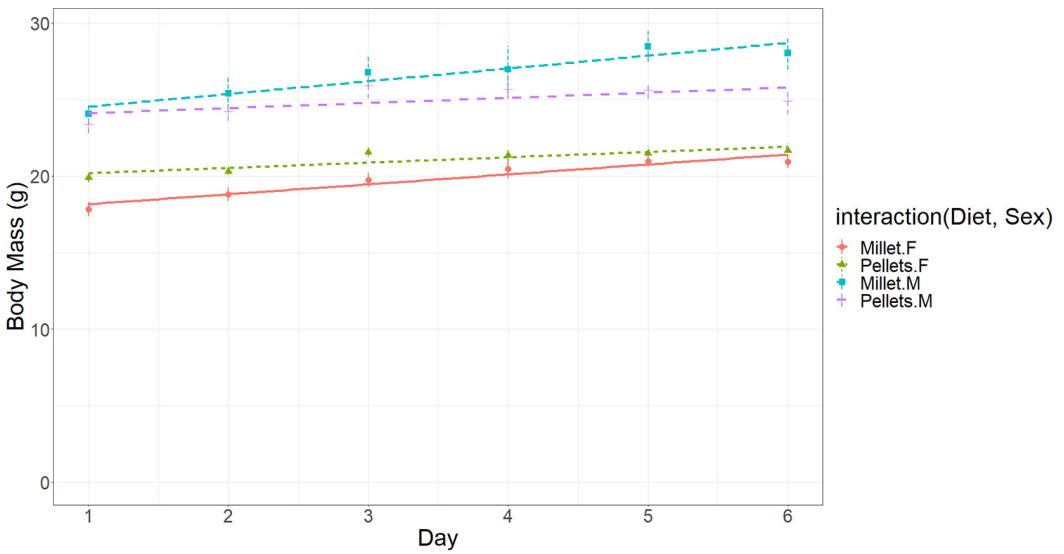


**FIGURE 7 |** Body mass (g) of the mice in the diet experiment for cancer mice fed a diet of either Millet or Mouse Chow Pellets. Error bars represent standard errors of the means.

At the end of the experiment there was some to no correlation across mice between the tumors on the right and left flanks [Pearson's correlation of 0.526 and −0.017 for the first ($n = 34$) and second ($n = 24$) experiments, respectively].

## DISCUSSION

Our goals were to: (1) determine whether one could conduct experiments with a mouse model under free range conditions, (2) measure effects of cancer burden on foraging metrics, (3) compare tumor growth rates with laboratory housed mice, and (4) begin to sort out confounding factors such as diet.

### Effects of Microhabitat, Sex, and Cancer on Foraging Behaviors

With respect to (1), laboratory mice, despite their pedigree, when released into the vivarium behaved as expected of wild rodents (e.g., Kotler et al., 1991). They sought shelter, they explored their environment, they searched for food and water, they dug through sandy substrates to harvest the millet seeds, and they traded off food and safety when making foraging decisions. They responded

strongly to the risk factor of microhabitat and recognized the open microhabitat as intrinsically more dangerous than sheltered areas under bushes as reflected in their GUDs. Thus C57BL/6 mice free-ranging in the vivarium show reasonable behavior that is similar to wild rodents and so offer a potential mouse model for more complex and complete lifestyles.

With respect to (2), cancer in the C57BL/6 laboratory mice gave rise to measurable changes in foraging patterns. Over time, free-range mice with cancer harvested more food yet saw a loss in body mass relative to those without cancer. Based on models of patch use and foraging economics, we interpret this as the cancer mice having a greater need for energy (higher marginal value of energy) or perceiving less to live for (lower survivor's fitness) as their tumors grew (Brown, 1992; Brown et al., 1997). Furthermore, mice with cancer became warier relative to the cancer-free mice. With time, mice with cancer harvested a greater and greater fraction of their food from the bush (safe) than open (risky) microhabitat relative to the cancer-free mice (note: both groups showed a general temporal tendency of harvesting more from the open).

Upon approaching a threshold tumor size, the mice were euthanized according to standard animal care protocols (for consistency, we euthanized all mice in Experiment 1 at the same time even though tumors were substantially smaller in the free-range than facility-housed mice). At that 30-day point, the free-range cancer mice continued to be highly active and forage extensively. They were not symptomatic to the extent that their foraging aptitudes were impaired or that they were debilitated with cachexia (body wasting). Presumably, had the tumors continued to grow, at some point the cancer mice's GUDs would have increased, either through an increase in foraging time or from the symptomatic effects of the tumor burden.

Regarding cancer in natural populations, the increased foraging effort and weight loss seen in the free-range cancer mice might render such animals less successful at reproducing and competing for food, and more susceptible to predators. Overall, we would thus expect cancerous animals to disappear more rapidly from the population. Thus, we might expect cancer to be rarely observed in natural populations (Ewald and Ewald, 2017; Madsen et al., 2017) even beyond the direct mortality caused by the disease itself.

Because cancer induces changes in foraging metrics (GUDs and habitat selection) and because cancer induces changes in body condition, this may allow for an animal model for evaluating quality of life. With the free-ranging mice, one can objectively define critical points such as when GUDs or habitat choices begin to change mice with growing tumor burden relative to mice without. At what level of cancer progression do GUDs begin to increase and effective foraging cease? How quickly do these change manifest with cancer cell lines that vary in metastatic potential and potential to induce cachexia? Finally, how does therapy alter foraging behavior? We suggest that the vivarium-based system described here when coupled with various types of cancer and targeted therapies can provide quantitative metrics of quality of life.

We envisage that we can investigate quality of life issues quantitatively using behavioral indicators (i.e., GUDs) by giving laboratory mice the appropriate cancer and the appropriate therapy drug regime in a full factorial design. The experimental design would consist of: the baseline for healthy mice (cancer-free, no therapy drugs; expectation of low GUDs); the negative effects of cancer on quality of life (plus cancer, no therapy drugs — this experiment; expectation of first lower, and then higher GUDs that track the energetic demands and the deleterious impact of cancer); the negative effects of the therapy on quality of life (cancer-free, plus therapy drugs; expectation of higher GUDs reflecting the adverse effect of the therapy on quality of life); and the combined effect of both cancer and therapy drugs in potentially increasing the quality of life (plus cancer, plus therapy drugs; therapy worthwhile so long as GUDs are lower than for cancer alone or therapy alone). The behavioral indicators can then be linked to physiological indicators as well. The mice without cancer reveal in their GUDs how they would like to go about their foraging (among the most significant activities for a non-human mammal) vs. how they do go about their business when burdened with cancer and/or therapy.

## Effect of Free-Ranging on Tumor Growth Rates

With respect to (3), we were able to demonstrate significantly lower tumor growth rates in the free-range mice relative to those housed in laboratory facilities. This is an intriguing result, that while not definitive in itself, shows the opportunity to track the growth of tumors in animals experiencing different ecological settings (Loizides et al., 2015). Differences could have been due to a multitude of factors including activity levels, stress, food intake, diet, and daily vicissitudes of weather and other stimuli, all factors known to or likely to influence tumor growth rates. While beyond our scope and resources, the present experiments show promise. Additional experiments could include mice housed in cages and placed in the vivarium so as to be exposed to the daily and nightly changes in climate and soundscape. Mice could be allowed to free-range in the vivarium, but have effort-free, *ad lib* access to food and water as is typical in the laboratory and some vivarium experiments, while mice in laboratory cages (sufficiently large, but still much smaller than a quadrant of the vivarium) would obtain their food from the seed trays used in our vivarium experiments. These experiments and more become intriguing and relevant in light of our results.

Regardless of husbandry, tumors were larger in males than females (Clocchiatti et al., 2016; Kim et al., 2018). The 4NQO cancer cell line was derived from a male mouse (Badarni et al., 2019). Its establishment at a larger size in males than females may reflect a boost from androgens (e.g., Birrell et al., 1995; Trigunaite et al., 2015). Beyond that, in humans, males and females often show different incidences of cancer, different outcomes from therapy, and different mortality rates (Clocchiatti et al., 2016). These may be due to different sensitivities of the cancer cells to estrogens and androgens, the role of sex chromosomes (Birrell et al., 1995), or the manner in which androgens suppress the immune system (Trigunaite et al., 2015), among other possible causes.

### Effect of Diet on Tumor Growth Rates

With respect to (4), we began to untangle the effects of diet and free-ranging on tumor growth rates. In the laboratory, we showed that tumor growth rates were higher for mice fed on millet than those fed on laboratory chow. This result does begin to rule out the role of diet in explaining why the free-range mice (fed millet) exhibited slower tumor growth rates than the mice housed in the laboratory facilities (fed on chow). As future directions, seeds or pelletized food can be varied or experimentally manipulated to have different nutritional compositions (Nersesian et al., 2011). One could then observe the effects of different foods on the foraging behaviors (e.g., Schmidt et al., 1998; Shrader et al., 2008) and tumor growth rates of the free-ranging C57BL/6 or even other mice strains relevant to cancer research.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The animal study was reviewed and approved by the Animal care and Ethics Committee of Ben-Gurion University.

## AUTHOR CONTRIBUTIONS

BK, DM, and JB conceived the project and designed the vivarium experiments. DM and EA conducted the vivarium experiments. ME and MP developed the cancer cell line and with EA and FS managed the laboratory experiments. DM, JM, EA, and JB managed data analyses. BK oversaw the entire project and wrote the initial manuscript draft. All authors contributed to edits and revisions.

## FUNDING

This work was funded by the Israel Science Foundation (Grants 259-19 to BK and 700/16 to ME). JB acknowledges funding from NIH/NCI 1U54CA193489-01A1, "Cancer as a Complex Adaptive System," NIH/NCI U54 Supplement, "The Tumor-host Evolutionary Arms Race".

## ACKNOWLEDGMENTS

We thank Liran Sagi for assistance with the initial data analysis and for writing codes that helped track the mice during the experiment. We also thank Orna Herling for helping record data. This work was carried out with the approval of the Ben-Gurion University Animal Care Committee, permit IL-08-01-2020(E).

## REFERENCES

Abramsky, Z., Rosenzweig, M. L., and Pinshow, B. (1991). The shape of a gerbil isocline measured using principles of optimal habitat selection. *Ecology* 72, 329–340. doi: 10.2307/1938926

Abramsky, Z., Rosenzweig, M. L., and Subach, A. (1997). Gerbils under threat of owl predation: isoclines and isodars. *Oikos* 78, 81–90. doi: 10.2307/3545803

Arcis, V., and Desor, D. (2003). Influence of environment structure and food availability on the foraging behaviour of the laboratory rat. *Behav. Processes* 60, 191–198. doi: 10.1016/s0376-6357(02)00122-5

Badarni, M., Prasad, M., Balaban, N., Zorea, J., Yegodayev, K. M., Ben-Zion, J., et al. (2019). Repression of AXL expression by AP-1/JNK blockage overcomes resistance to PI3Ka therapy. *JCI Insight* 4, e125341.

Bär, J., Leung, J. M., Hansen, C., Loke, P. N., Hall, A. R., Conour, L., et al. (2020). Strong effects of lab-to-field environmental transitions on the bacterial intestinal microbiota of Mus musculus are modulated by *Trichuris muris* infection. *FEMS Microbiol. Ecol.* 96:fiaa167.

Berger-Tal, O., and Kotler, B. P. (2010). State of emergency: gerbil behavior is affected by the hunger state of their predators. *Ecology* 93, 593–600.

Berger-Tal, O., Kotler, B. P., Mukherjee, S., and Brown, J. S. (2010). Complex state-dependent games between owls and gerbils. *Ecol. Lett.* 13, 302–310. doi: 10.1111/j.1461-0248.2010.01447.x

Betof, A. S., Lascola, C. D., Weitzel, D., Landon, C., Scarbrough, P. M., Devi, G. R., et al. (2015). Modulation of murine breast tumor vascularity, hypoxia, and chemotherapeutic response by exercise. *J. Nat. Cancer Inst.* 107:djv040.

Birrell, S. N., Bente, J. M., Hickey, T. E., Ricciardelli, C., Weger, M. A., Horsfall, D. J., et al. (1995). Androgens induce divergent proliferative responses in human breast cancer cell lines. *J. Steroid Biochem. Mol. Biol.* 52, 459–467. doi: 10.1016/0960-0760(95)00005-k

Boursot, P., Auffray, J. C., Britton-Davidian, J., and Bonhomme, F. (1993). The evolution of house mice. *Ann. Rev. Ecol. Syst.* 24, 119–152.

Brown, J. S. (1989). Desert rodent community structure: a test of four mechanisms of coexistence. *Ecol. Monogr.* 59, 1–20.

Brown, J. S. (1988). Patch use as an indicator of habitat preference, predation risk, and competition. *Behav. Ecol. Sociobiol.* 22,37–47.

Brown, J. S. (1992). Patch use under predation risk: I. Models and predictions. *Annal. Zool. Fenni.* 29, 301–309.

Brown, J. S., Kotler, B. P., and Mitchell, W. A. (1997). Competition between birds and mammals: a comparison of giving-up densities between crested larks and gerbils. *Evol. Ecol.* 11, 757–772. doi: 10.1023/a:1018442503955

Brown, J. S., Kotler, B. P., Smith, R. J., and Wirtz, W. O. II (1988). The effects of owl predation on the foraging behavior of heteromyid rodents. *Oecologia* 76, 408–415. doi: 10.1007/bf00377036

Chung, H. Y., and Park, Y. K. (2017). Rationale, feasibility and acceptability of ketogenic diet for cancer treatment. *J. Cancer Prevent.* 22:127.

Clarke, J. A. (1983). Moonlight's influence on predator/prey interactions between short-eared owls (*Asio flammeus*) and deermice (*Peromyscus maniculatus*). *Behav. Ecol. Sociobiol.* 13, 205–209. doi: 10.1007/bf00299924

Clocchiatti, A., Cora, E., Zhang, Y., and Paolo Dotto, G. (2016). Sexual dimorphism in cancer. *Nat. Rev. Cancer* 16, 330–339.

Cope, E. C., Opendak, M., LaMarca, E. A., Murthy, S., Park, C. Y., Olson, L. B., et al. (2019). The effects of living in an outdoor enclosure on hippocampal plasticity and anxiety-like behavior in response to nematode infection. *Hippocampus* 29, 366–377. doi: 10.1002/hipo.23033

Cornwall, D. H., Ruff, J. S., Zachary, E. R., Young, C. P., Maguire, K. M., Painter, R. J., et al. (2021). Horizontal transmission of a murine retrovirus is driven by males within semi-natural enclosures. *Funct. Ecol.* 35, 884–893. doi: 10.1111/1365-2435.13766

Cucchi, T., and Vigne, J. D. (2006). Origin and diffusion of the house mouse in the Mediterranean. *Hum. Evol.* 21, 95–106. doi: 10.1007/s11598-006-9011-z

Cucchi, T., Papayianni, K., Cersoy, S., Aznar-Cormano, L., Zazzo, A., Debruyne, R., et al. (2020). Tracking the Near Eastern origins and European dispersal of the western house mouse. *Sci. Rep.* 10, 1–12.

Dice, L. R. (1945). Minimum intensities of illumination un-der which owls can find dead prey by sight. *Am. Nat.* 79, 385–416. doi: 10.1086/281276

Embar, K., Kotler, B. P., Mukherjee, S., and Brown, J. S. (2011). Risk management in optimal foragers: the effect of sightlines and predators on patch use, time

allocation, vigilance, and apprehension in gerbils. *Oikos* 120, 1657–1666. doi: 10.1111/j.1600-0706.2011.19278.x

Ewald, P. W., and Ewald, H. A. S. (2017). "Infection and cancer in nature," in *Ecology And Evolution Of Cancer*, eds B. Ujvari, B. Roche, and F. Thomas (London: Academic Press), 47–56. doi: 10.1016/b978-0-12-804310-3.00003-x

Festing, M. F. (1979). Properties of inbred strains and outbred stocks, with special reference to toxicity testing. *J. Toxicol. Environ. Health* 5, 53–68. doi: 10.1080/15287397909529725

Gaukler, S. M., Ruff, J. S., Galland, T., Underwood, T. K., Kandaris, K. A., Liu, N. M., et al. (2016a). Quantification of cerivastatin toxicity supports organismal performance assays as an effective tool during pharmaceutical safety assessment. *Evol. Appl.* 9, 685–696. doi: 10.1111/eva.12365

Gaukler, S. M., Ruff, J. S., and Potts, W. K. (2016b). Paroxetine exposure skews litter sex ratios in mice suggesting a Trivers–Willard process. *Behav. Ecol.* 27, 1113–1121. doi: 10.1093/beheco/arw017

Graham, A. L. (2021). Naturalizing mouse models for immunology. *Nat. Immunol.* 22, 111–117. doi: 10.1038/s41590-020-00857-2

Grimm, M. S., Emerman, J. T., and Weinberg, J. (1996). Effects of social housing condition and behavior on growth of the Shionogi mouse mammary carcinoma. *Physiol. Behav.* 59, 633–642. doi: 10.1016/0031-9384(95)02126-4

Hawkins, B. L., Heniford, B. L., Ackermann, D. M., Leonberger, M., Martinez, S. A., and Hendler, F. J. (1994). 4NQO carcinogenesis: a mouse model of oral cavity squamous cell carcinoma. *Head Neck* 16, 424–432. doi: 10.1002/hed.2880160506

Ho, V. W., Leung, K., Hsu, A., Luk, B., Lai, J., Shen, S. Y., et al. (2011). A low carbohydrate, high protein diet slows tumor growth and prevents cancer initiation. *Cancer Res.* 71, 4484–4493. doi: 10.1158/0008-5472.can-10-3973

Idorn, M., and Thor Straten, P. (2017). Exercise and cancer: from "healthy" to "therapeutic"? *Cancer Immunol., Immunother.* 66, 667–671.

Jones, L. W., Antonelli, J., Masko, E. M., Broadwater, G., Lascola, C. D., Fels, D., et al. (2012). Exercise modulation of the host-tumor interaction in an orthotopic model of murine prostate cancer. *J. Appl. Physiol.* 113, 263–272. doi: 10.1152/japplphysiol.01575.2011

Karp, N. A., Wilson, Z., Stalker, E., Mooney, L., Lazic, S. E., Zhang, B., et al. (2020). A multi-batch design to deliver robust estimates of efficacy and reduce animal use–a syngeneic tumour case study. *Sci. Rep.* 10, 1–10.

Kim, H., Lim, H., and Moon, A. (2018). Sex differences in cancer: epidemiology, genetics and therapy. *Biomol. Ther.* 26, 335–342. doi: 10.4062/biomolther.2018.103

Kokolus, K. M., Capitano, M. L., Lee, C. T., Eng, J. W. L., Waight, J. D., Hylander, B. L., et al. (2013). Baseline tumor growth and immune control in laboratory mice are significantly influenced by subthermoneutral housing temperature. *Proc. Natl. Acad. Sci. U.S.A.* 110, 20176–20181. doi: 10.1073/pnas.1304291110

Kotler, B. P. (1997). Patch use by gerbils in a risky environment: manipulating food and safety to test four models. *Oikos* 78, 274–282.

Kotler, B. P., and Blaustein, L. (1995). Titrating food and safety in a heterogeneous environment: when is the risky patch of equal value? *Oikos* 74, 251–258.

Kotler, B. P., Brown, J. S., and Bouskila, A. (2004). Apprehension and time allocation in gerbils: the effects of predatory risk and energetic state. *Ecology* 85, 917–922. doi: 10.1890/03-3002

Kotler, B. P., Brown, J. S., and Hasson, O. (1991). Factors affecting gerbil foraging behavior and rates of owl predation. *Ecology* 72, 2249–2260.

Kotler, B. P., Brown, J. S., Bleicher, S. S., and Embar, K. (2016). Intercontinental-wide consequences of compromise-breaking adaptations: the case of desert rodents. *Israel J. Ecol. Evol.* 62, 186–195. doi: 10.1080/15659801.2015.1125832

Kotler, B. P., Brown, J. S., Mukherjee, S., Berger-Tal, O., and Bouskila, A. (2010). Moonlight avoidance in gerbils reveals a sophisticated interplay among time allocation, vigilance, and state-dependent foraging. *Proc. R. Soc. Lond. B.* 277, 1469–1474. doi: 10.1098/rspb.2009.2036

Kotler, B. P., Brown, J. S., Smith, R. J., and Wirtz, W. O. I. I. (1988). The effects of morphology and body size on the rates of predation in desert rodents. *Oikos* 53, 145–152. doi: 10.2307/3566056

Lee, C., Raffaghello, L., Brandhorst, S., Safdie, F. M., Bianchi, G., Martin-Montalvo, A., et al. (2012). Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. *Sci. Translat. Med.* 4:124ra27. doi: 10.1126/scitranslmed.3003293

Leung, J. M., Budischak, S. A., Chung The, H., Hansen, C., Bowcutt, R., Neill, R., et al. (2018). Rapid environmental effects on gut nematode susceptibility in rewilded mice. *PLoS Biol.* 16:e2004108. doi: 10.1371/journal.pbio.2004108

Loizides, C., Iacovides, D., Hadjiandreou, M. M., Rizki, G., Achilleos, A., Strati, K., et al. (2015). Model-based tumor growth dynamics and therapy response in a mouse model of de novo carcinogenesis. *PLoS One* 10:e0143840. doi: 10.1371/journal.pone.0143840

Longland, W. S., and Price, M. V. (1991). Direct observations of owls and heteromyid rodents: can predation risk explain microhabitat use? *Ecology* 72, 2261–2273.

Madsen, T., Arnal, A., Vittecoq, M., Bernex, F., Abadie, J., Labrut, S., et al. (2017). "Cancer prevalence and etiology in wild and captive animals," in *Ecology And Evolution Of Cancer*, eds B. Ujvari, B. Roche, and F. Thomas (London: Academic Press), 9–46.

Makin, D. F., Kotler, B. P., Brown, J. S., Garrido, M., and Menezes, J. F. S. (2020). The enemy within: how does a bacterium inhibit the foraging aptitude and risk management behavior of Allenby's gerbils? *Am. Nat.* 196, 717–729. doi: 10.1086/711397

Meagher, S., Penn, D. J., and Potts, W. K. (2000). Male–male competition magnifies inbreeding depression in wild house mice. *Proc. Natl. Acad. Sci. U.S.A.* 97, 3324–3329.

Morris, J. S., Ruff, J. S., Potts, W. K., and Carrier, D. R. (2017). A disparity between locomotor economy and territory-holding ability in male house mice. *J. Exp. Biol.* 220, 2521–2528.

Mowry, A. V., Donoviel, Z. S., Kavazis, A. N., and Hood, W. R. (2017). Mitochondrial function and bioenergetic trade-offs during lactation in the house mouse (*Mus musculus*). *Ecol. Evol.* 7, 2994–3005.

Nencioni, A., Caffa, I., Cortellino, S., and Longo, V. D. (2018). Fasting and cancer: molecular mechanisms and clinical application. *Nat. Rev. Cancer* 18, 707–719. doi: 10.1038/s41568-018-0061-0

Nersesian, C. L., Banks, P. B., and McArthur, C. (2011). Titrating the cost of plant toxins against predators: determining the tipping point for foraging herbivores. *J. Anim. Ecol.* 80, 753–760. doi: 10.1111/j.1365-2656.2011.01822.x

O'Neill, A. M., Burrington, C. M., Gillaspie, E. A., Lynch, D. T., Horsman, M. J., and Greene, M. W. (2016). High-fat Western diet–induced obesity contributes to increased tumor growth in mouse models of human colon cancer. *Nutr. Res.* 36, 1325–1334.

Parohan, M., Sadeghi, A., Khatibi, S. R., Nasiri, M., Milajerdi, A., Khodadost, M., et al. (2019). Dietary total antioxidant capacity and risk of cancer: a systematic review and meta-analysis on observational studies. *Crit. Rev. Oncol. Hemat.* 138, 70–86.

Phifer-Rixey, M., Bi, K., Ferris, K. G., Sheehan, M. J., Lin, D., Mack, K. L., et al. (2018). The genomic basis of environmental adaptation in house mice. *PLoS Genet.* 14:e1007672. doi: 10.1371/journal.pgen.1007672

Rao, G. N., Birnbaum, L. S., Collins, J. J., Tennant, R. W., and Skow, L. C. (1988). Mouse strains for chemical carcinogenicity studies: overview of a workshop. *Toxicol. Sci.* 10, 385–394. doi: 10.1016/0272-0590(88)90285-0

Rohner, C., and Krebs, C. J. (1996). Owl predation on snowshoe hares: consequences of antipredator behaviour. *Oecologia* 108, 303–310. doi: 10.1007/bf00334655

Rose, M. L., Madren, J., Bunzendahl, H., and Thurman, R. G. (1999). Dietary glycine inhibits the growth of B16 melanoma tumors in mice. *Carcinogenesis* 20, 793–798. doi: 10.1093/carcin/20.5.793

Ruff, J. S., Cornwall, D. H., Morrison, L. C., Cauceglia, J. W., Nelson, A. C., Gaukler, S. M., et al. (2017). Sexual selection constrains the body mass of male but not female mice. *Ecol. Evol.* 7, 1271–1275. doi: 10.1002/ece3.2753

Ruff, J. S., Suchy, A. K., Hugentobler, S. A., Sosa, M. M., Schwartz, B. L., Morrison, L. C., et al. (2013). Human-relevant levels of added sugar consumption increase female mortality and lower male fitness in mice. *Nat. Commun.* 4, 1–7.

Schmidt, K. A., Brown, J. S., and Morgan, R. A. (1998). Plant defenses as complementary resources: a test with squirrels. *Oikos* 81, 130–142. doi: 10.2307/3546475

Shrader, A. M., Kotler, B. P., Brown, J. S., and Kerley, G. I. H. (2008). Providing water for goats in arid landscapes: effects on feeding effort with regard to time period, herd size and secondary compounds. *Oikos* 117, 466–472.

Song, H. K., and Hwang, D. Y. (2017). Use of C57BL/6N mice on the variety of immunological researches. *Lab. Anim. Res.* 33, 119–123. doi: 10.5625/lar.2017.33.2.119

Thaker, P. H., Han, L. Y., Kamat, A. A., Arevalo, J. M., Takahashi, R., Lu, C., et al. (2006). Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat. Med.* 12, 939–944.

Trigunaite, A., Dimo, J., and Jørgensen, T. N. (2015). Suppressive effects of androgens on the immune system. *Cell Immun.* 294, 87–94. doi: 10.1016/j.cellimm.2015.02.004

Troxell-Smith, S. M., Tutka, M. J., Albergo, J. M., Balu, D., Brown, J. S., and Leonard, J. P. (2016). Foraging decisions in wild versus domestic *Mus musculus*: what does life in the laboratoryselect for? *Behav. Proc.* 122, 43–50. doi: 10.1016/j.beproc.2015.10.020

Wang, Y., Corr, J. G., Thaler, H. T., Tao, Y., Fair, W. R., and Heston, W. D. (1995). Decreased growth of established human prostate LNCaP tumors in nude mice fed a low-fat diet. *J. Nat. Cancet Inst.* 87, 1456–1462. doi: 10.1093/jnci/87.19.1456

Weber, D. D., Aminzadeh-Gohari, S., Tulipan, J., Catalano, L., Feichtinger, R. G., and Kofler, B. (2020). Ketogenic diet in the treatment of cancer–where do we stand? *Mol. Metab.* 33, 102–121. doi: 10.1016/j.molmet.2019.06.026

Whelan, C. J., Brown, J. S., and Hank, A. E. (2015). Diet preference in the house sparrow asser domesticus: hooked on millet? *Bird Study* 62, 569–573.

Yeung, F., Chen, Y. H., Lin, J. D., Leung, J. M., McCauley, C., Devlin, J. C., et al. (2020). Altered immunity of laboratory mice in the natural environment is associated with fungal colonization. *Cell Host Microbe* 27, 809–822.

Zielinski, M. R., Muenchow, M., Wallig, M. A., Horn, P. L., and Woods, J. A. (2004). Exercise delays allogeneic tumor growth and reduces intratumoral inflammation and vascularization. *J. Appl. Physiol.* 96, 2249–2256. doi: 10.1152/japplphysiol.01210.2003

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