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Social evolution and the what, when, why and how of the major evolutionary transitions in the history of life

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Social evolution and the what, when, why and how of the major evolutionary transitions in the history of life

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Editorial: Social evolution and the what, when, why and how of the major evolutionary transitions in the history of life

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Editorial on the Research Topic
Social evolution and the what, when, why and how of the major evolutionary transitions in the history of life

In their foundational book, Maynard Smith and Szathmáry (1995) proposed eight rare events in the history of life on Earth that were transformative and seemingly needed to overcome significant selective barriers to their evolution and spread. These were called Major Evolutionary Transitions, or METs. Although subsequently this list of METs has been amended, edited, narrowed, and expanded by a variety of authors (e.g., Bourke, 2011; Calcott and Sterelny, 2011; Szathmáry, 2015; West et al., 2015; Herron, 2021), we still lack consensus regarding the defining qualities of METs in terms of their evolutionary and ecological consequences. In this Research Topic, we revisit these issues to synthesize new research and novel ideas that may illuminate the ecological and evolutionary conditions that give rise to and result from the METs. Some authors broadly consider what constitutes a MET (Robin et al.; Okasha). Others provide novel insights into particular METs such as multicellularity, eusociality, mutualisms in the microbiome, and even identify previously overlooked transitions among insect endosymbionts that seem to track the same evolutionary path that gave rise to mitochondria (Rose and Hammerschmidt; Bernadou et al.; da Silva; Zachar and Boza; Rafiqi et al.). Finally, several propose fresh considerations of the evolutionary processes that facilitate METs (Thies and Watson; Lamm and Kolodny; Watson et al.).

Despite decades of debate, there is no clear consensus about what is or produces a MET. For example, ecological effects often are excluded in defining METs (e.g., Szathmáry, 2015). Nevertheless, an apparent large ecological impact correlated with a MET seems to be at least an implicit criterion. Would, for example, the origin of eukaryotes rank as a "major" event if eukaryotes had remained minor components in a prokaryotic world, as they were for the first billion years of their existence? Therefore, Robin et al. examine how METs do or do not factor into Major System Transitions (MSTs) of entire ecosystems. Viewed in this way, a MST can result from METs that produce gains in information availability and storage capacity for organisms (i.e., through diploid genomes, learning recorded in neurons, or symbolic language etched into abiotic media), combining with METs in which previously independent entities fuse to become a single, integrated individual. Lamm and Kolodny further consider how populations can create novel and usable information as a distributed adaptation that is essential for individual success. Such adaptations do not exist within any individual and are only evident when observing the features of groups or populations. These papers follow Maynard Smith and Szathmáry (1995) original proposal that METs can reflect both changes in information and individuality. Alternatively, Okasha argues that unifying the concept of a MET is better served by excluding informational changes, and thus narrowing the definition to changes in individuality. What remains is a philosophical issue that requires differentiating across conceptual and ontological questions. The former includes the types of explanations and modeling approaches applied to METs, while the latter includes the nature and hierarchical organization of biological entities. In both categories, Okasha identifies areas where empirical science and philosophical analyses fruitfully overlap.

One such ontological question concerns the mechanisms underlying the origins of complex insect societies as a possible MET. This is addressed by da Silva showing that there may exist differing precursors to eusociality: semisociality (cooperating females of the same generation) and subsociality (cooperating mothers and daughters) for wasps and bees, respectively. Staying with insect societies, Bernadou et al. posit that worker sterility is key to allowing reproductives to simultaneously increase in fecundity and lifespan. Breaking this trade-off is what produces a "superorganism", which they propose restricts the potential for a MET to only those insect societies with obligately sterile workers. Rafiqi et al. expand the general consideration of how a cooperative entity can evolve by using endosymbiotic evolution within insects as a model system for the role of development in integration of separate species into a single entity with aligned fitness outcomes. Finally, Rose and Hammerschmidt emphasize the importance of differentiating levels of multicellularity. They propose that the pathway to a MET follows three stages: individuals forming groups; groups acting as individuals; and entities subsuming their individuality into a singular organism. Different questions and processes are relevant across these stages.

The Research Topic also adds to the conceptual consideration of general theories for the evolution of METs (Okasha). Zachar and Boza raise the paradox of why there have been relatively few (or no) surviving symbioses among prokaryotes comparable to the one that produced mitochondria. They examine why mutualisms do not lead to multilevel selection more often in microbial communities, resulting in community-wide inheritance and heritable multispecies phenotypes. While there is no theoretical objection for a multispecies community evolving to a superorganism, the lack of such events indicates that there may be only one way for microbes to make a major transition in individuality: endosymbiosis.

Changes in levels of selection are considered a critical MET characteristic. However, formal approaches to quantifying group selection (contextual analysis and the Price approach), can give contradictory answers. Distinguishing among causes of this discrepancy requires comparison of contrasting treatments/experimental interventions, which no statistical analysis of a single treatment can provide (Thies and Watson). Watson et al. then ask how functional relationships need to be organized to create fitness differences that properly belong to a collective and not its parts. Connectionist models of learning and cognition may identify formally non-decomposable collective phenotypes, providing this critical feature of METs.

In summary, the Research Topic examines and provides new insights into a range of what, when, why and how questions about METs. It opens new avenues for thinking about when in the history of life major events became possible, which events have profoundly altered the world, what those events required, and how and why they could arise through selective and evolutionary processes.

Author contributions

All authors contributed to the writing and editing of the manuscript and approved the submitted version.

Conflict of interest

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Major Evolutionary Transitions in Social Insects, the Importance of Worker Sterility and Life History Trade-Offs

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The evolution of eusociality in social insects, such as termites, ants, and some bees and wasps, has been regarded as a major evolutionary transition (MET). Yet, there is some debate whether all species qualify. Here, we argue that worker sterility is a decisive criterion to determine whether species have passed a MET (= superorganisms), or not. When workers are sterile, reproductive interests align among group members as individual fitness is transferred to the colony level. Division of labour among cooperating units is a major driver that favours the evolution of METs across all biological scales. Many METs are characterised by a differentiation into reproductive versus maintenance functions. In social insects, the queen specialises on reproduction while workers take over maintenance functions such as food provisioning. Such division of labour allows specialisation and it reshapes life history trade-offs among cooperating units. For instance, individuals within colonies of social insects can overcome the omnipresent fecundity/longevity trade-off, which limits reproductive success in organisms, when increased fecundity shortens lifespan. Social insect queens (particularly in superorganismal species) can reach adult lifespans of several decades and are among the most fecund terrestrial animals. The resulting enormous reproductive output may contribute to explain why some genera of social insects became so successful. Indeed, superorganismal ant lineages have more species than those that have not passed a MET. We conclude that the release from life history constraints at the individual level is a important, yet understudied, factor across METs to explain their evolutionary success.

Keywords: ants, bees, life history trade-off, major evolutionary transitions, social evolution, superorganism, social insects, termites

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INTRODUCTION

Life on earth has evolved through rare but large steps called major evolutionary transitions (METs; Maynard Smith and Szathmáry, 1995; Michod, 1997; Bourke, 2011; West et al., 2015). During such a transition, organisation is shifted. Individual units (e.g., cells or insects) which were previously independent, integrate into a new larger "entity" (e.g., multicellular organisms or

superorganisms) (see **Table 1** for glossary of bold terms) (Maynard Smith and Szathmáry, 1995; Bourke, 2011; West et al., 2015; **Figure 1**). A MET has therefore been defined to be accomplished, when the fitness of the lower-level units is completely transferred to the higher level (see e.g., Okasha, 2005, 2006). As a consequence, the higher-level unit becomes the common unit of selection, evolutionary fitness interests among lower-level units are aligned and within-lower-level conflict becomes rare. The transition from multicellular organisms to eusocial animal societies, such as honey bee colonies, has been referred to as a MET (e.g., Maynard Smith and Szathmáry, 1995; Bourke, 2011, 2019; Szathmáry, 2015; West et al., 2015; Helanterä, 2016; Boomsma and Gawne, 2018). Yet debates exist whether they qualify as such. The large degree of social organisation that exists in social insects is a major reason for these discussions. Even the term **eusociality** covers considerable variation (e.g., Bourke, 1999, 2019; Anderson and McShea, 2001; Korb and Heinze, 2016; Boomsma and Gawne, 2018 and references therein). The variation in social organisation has been categorised into three steps that characterise a MET (e.g., Bourke, 2011; Korb and Heinze, 2016 for details). Using these three steps, we outline when we consider eusocial species as superorganismal (i.e., having passed a MET). We apply the theoretically founded criterion of complete fitness transfer to the higher level (Okasha, 2006). Thus, in our opinion, a MET is only realised in species with **sterile workers** (including soldiers; i.e., true neuters) because only then the lower-level fitness of colony members (lower-level units; i.e., workers/soldiers and queens as well as kings in termites) is completely transferred to the colony level (higher-level unit). This colony level fitness is realized by a colonies' queen/king, which represent the exclusive germline of these superorganisms. Workers can only increase their fitness by increasing the reproductive success of the colony, which means by supporting their germline, the queen/king, to which they are related. Under these conditions, the inclusive fitness of workers/soldiers consist exclusively of the indirect fitness component, which is the (direct) fitness of the higher evolutionary unit (i.e., the colony). Therefore, conflict over reproduction among group members is absent as reproductive interests are aligned. Based on this consideration, we highlight the importance of division of labour and specialisation among lower-level units in overcoming life history trade-offs as potential drivers toward METs.

TABLE 1 | Definition of terms used in the paper.

Term	Definition
Eusociality	Insects/animals that are characterised by overlapping generations, reproductive division of labour, and brood care.
Superorganisms	Eusocial insects/animals with sterile workers/soldiers, and accordingly a complete germline (queen/king) – soma (workers, soldiers) separation; in superorganisms the fitness of the individuals (lower-level units) is transferred to the colony (higher-level unit) realized *via* the colonies' germline, the queen (and sometimes also a king). Superorganims qualify as having passed a major evolutionary transition.
Sterile workers	Workers which have completely lost their reproductive organs. Alternatively, workers can be considered as sterile if their ovaries are no longer involved in reproduction but only in alternative functions.
Direct fitness	Number of own offspring (or better, allele copies) produced and transmitted to the next generation without the help of others; equivalent to classical Darwinian fitness.
Indirect fitness	Number of offspring (or better, allele copies) that relatives produce and transmit to the next generation due to the help of the altruist, weighed by relatedness.
By-product mutualism	A cost-free interaction between individuals/units from which all participants derive direct fitness benefits as by-products of the action of others.
Cooperation	A costly interaction between individuals/units from which all participants derive net direct fitness benefits.
Altruism	A costly interaction between individuals/units in which one partner, the altruist, increases the direct fitness of another individual, recipient, with net direct fitness costs for the altruist.
Evolutionary cheaters	Individuals/units, which invest less than their fair share into an association, thus exploiting their partners; selection generally favours such cheaters in the short–run, so that they often can "threaten" the stability of a cooperative interaction.
Fraternal transitions	Major evolutionary transitions that originated from associations of similar, related units; "more of the same" which results in division of labour after the association formed.
Egalitarian transitions	Major evolutionary transitions that originated from associations of different, disparate units, both partners reproduce; each unit contributed different functions right from the beginning to the association.

They are highlighted in bold, when first mentioned in the text.

THREE STEPS CHARACTERISE A MAJOR EVOLUTIONARY TRANSITION

Three steps characterise a MET (e.g., Bourke, 2011; Korb and Heinze, 2016 for details). These steps go along with important changes in terms of **cooperation** and conflict within groups (see Queller and Strassmann, 2009; **Figure 1A**):

(1) *Group formation (cooperation: intermediate, conflict: low).* The first stage, group formation, results from individuals coming together. It is selected mainly because individuals gain **direct fitness**, for instance, from selfish herd effects, improved protection against predators, facilitated resource exploitation, and/or energetic benefits as in the case of birds migrating in flocks. Taxon-specific ecology is important and different ecological conditions favour associations in different species and in different populations (e.g., Korb and Heinze, 2008; Jetz and Rubenstein, 2011; Rubenstein and Abbot, 2017 and reference therein). Strictly speaking, group formation is often a result of **by-product mutualisms** (sensu Bshary and Bergmüller, 2008) as no costs are involved for the participating individuals. Group formation is facilitated by a lack of local scale competition over resources or mating partners. Division of labour (DOL) plays a minor role and these associations are often transient. None of the eusocial animals belongs into this category as they live in non-transient groups.

(2) *Group maintenance (cooperation: increasing from intermediate to high, conflict: decreasing from intermediate*

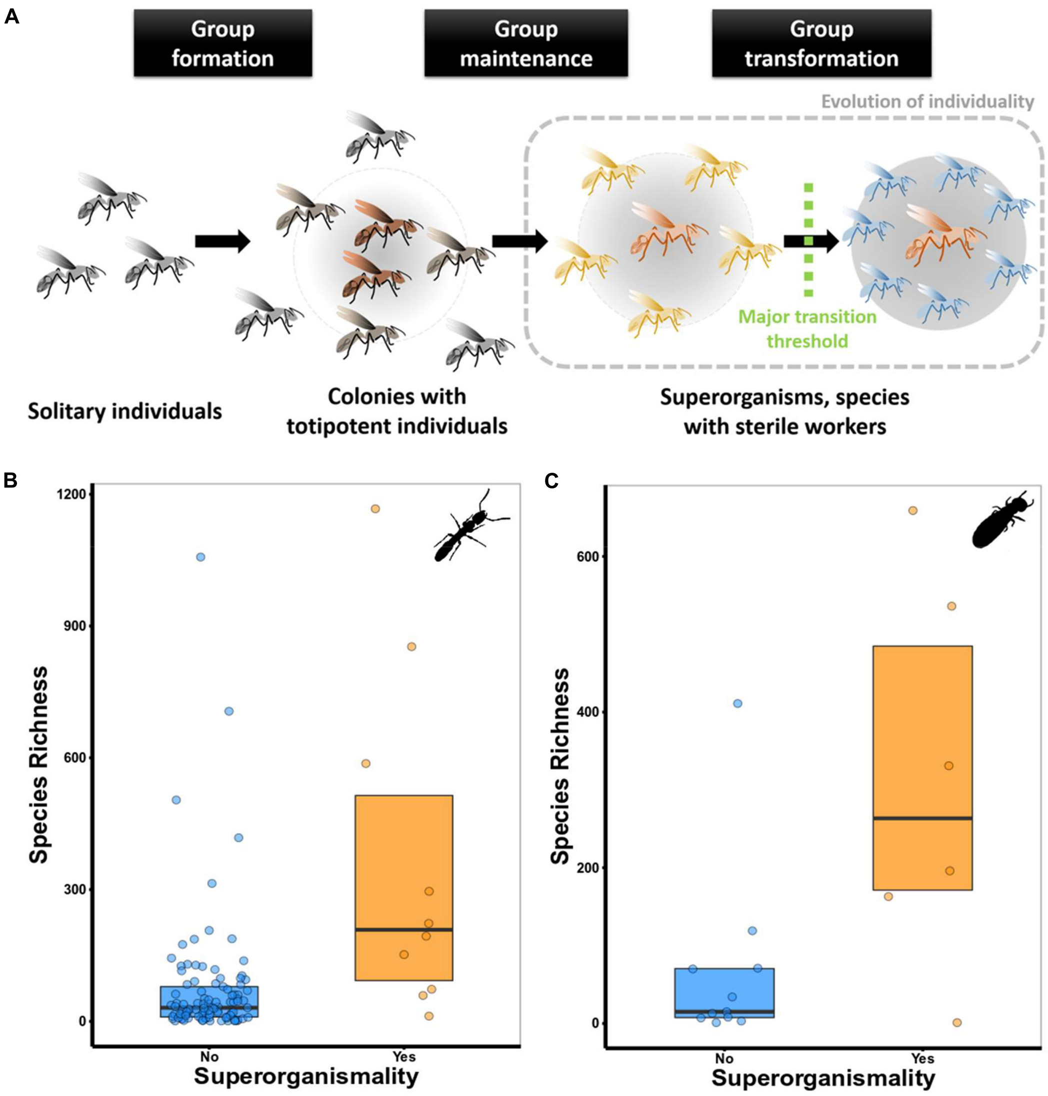

FIGURE 1 | (A) The three key steps that characterise a major evolutionary transition (following Bourke, 2011, 2019). These steps go along with important changes in terms of cooperation and conflict within groups (see main text for details). **(B)** Species richness in superorganismal and non-superorganismal ant genera. Species richness was significantly higher in superorganismal than non-superorganismal ant genera (Mann–Whitney U test: $N = 337$, $W = 355$, $P < 0.005$). Controlling for phylogenetic dependencies, a phylogenetic ANOVA was run on a subset of 111 genera for which a phylogeny was available. They showed that genera with sterile workers ($N = 10$) had higher species richness than genera that we do not consider superorganismal ($F = 24.56$, $P \leq 0.005$) (see **Supplementary Material** for more details). **(C)** Species richness in superorganismal and non-superorganismal termite lineages. There was a trend for species richness to be significantly higher in superorganismal than non-superorganismal termite lineages (Mann–Whitney U test: $N = 17$, $W = 13.5$, $P = 0.056$). The phylogenetic ANOVA revealed no differences in species richness between superorganismal ($N = 6$) and non-superorganismal ($N = 6$) termite lineages ($N = 12$, $F = 2.28$, $P = 0.371$).

to low). The second step, group maintenance, refers to established groups, which are not transient. Non-transient groups often arise through parent/offspring associations with extended brood care (i.e., subsociality) (Grosberg and Strathmann, 2007; Michod, 2007; Boomsma, 2009; Griesser et al., 2017). DOL appears as another major driver of the evolution of permanent groups (e.g., Michod, 2007). DOL results in complementary functions and/or traits, which increase the direct fitness of interacting partners. Continued selection of specialisation can lead to reciprocal dependence and further enhanced fitness (for detail, see below). Such permanent groups are generally characterised by cooperation rather than by-product mutualisms. Hence costs are involved and the evolutionary stability of groups can be threatened by **evolutionary cheaters**. Thus, the evolution of mechanisms of conflict resolution, such as partner choice or sanctioning of selfish individuals, is important for the evolutionary stability of these groups (e.g., Frank, 1998).

In the case of social insects (or more broadly, all **fraternal associations**), **indirect fitness** benefits derived from helping relatives are of major importance (e.g., Korb and Heinze, 2004; Foster et al., 2006; Boomsma, 2009; Abbot et al., 2011). They facilitate stable maintenance of associations, and potential selection against cheating, for instance through the evolution of self-restraint (e.g., Frank, 1998; Ratnieks et al., 2006 and references therein). In the case of the evolution of **altruism**, relatedness is even a necessary pre-requisite (Foster et al., 2006; Boomsma, 2009; Abbot et al., 2011). Importantly in all associations of step 2, single individuals still have the potential to produce own offspring (i.e., gain direct fitness benefits) so that fitness is not completely transferred from the lower to the higher level and conflict over reproduction among lower-level units remains.

Many systems of cooperatively breeding birds and mammals (Rubenstein and Abbot, 2017) can be classified as having crossed the group maintenance step. Among the eusocial animals, all those species, in which workers and/or soldiers (i.e., the altruistic castes) can still reproduce, belong into this category. This includes, e.g., social mole rats, many social aphids and thrips, all termite species except the Termitidae, and all social Hymenopterans in which workers can produce males (including the honey bee) (Korb and Heinze, 2016; see **Supplementary Table 1**).

(3) *Group transformation (cooperation: high; conflict: low).* The third stage, group transformation, generally considers groups that have passed a MET (Okasha, 2006; Bourke, 2011; West et al., 2015; Boomsma and Gawne, 2018). Formally, it corresponds to the emergence of new higher-level entities (here, groups composed of individuals) (Okasha, 2006; Bourke, 2011; West et al., 2015). Most researchers would agree that social insects in the third stage are superorganisms. Yet, disagreement and debates remain about the strict criterion or thresholds at which a new higher level entity emerges. To date, mostly definitions are favoured which centre around the loss of totipotency of individuals (e.g., Boomsma and Gawne, 2018) or which are based on a continuum of the extent of cooperation and conflict (Queller and Strassmann, 2009) (for a discussion, see below). In the current paper, we strictly apply the theoretically founded ultimate criterion of complete transfer of fitness of lower-units to the higher-level unit (see above).

Accordingly, in our opinion, in social insects, group transformation is reached when workers are sterile, so that they cannot gain any direct fitness. We consider worker sterility to be reached when workers have completely lost their reproductive organs or when ovaries are no longer involved in reproduction but "only" necessary for other physiological or developmental processes (Khila and Abouheif, 2010). Under such conditions, workers cannot gain any direct fitness, but only indirect fitness *via* the reproductive success of the colony. The colony is the unit of selection and the indirect fitness of its members becomes the direct fitness of the colony. Social insects with sterile workers qualify as "true" superorganisms with a complete separation of the germline (queen, and in termites also a king) and the soma (workers and sometimes soldiers) (Korb and Heinze, 2016).

From this perspective, surprisingly few eusocial insect species have passed the MET - and no eusocial mammal – all of which qualify in analogy of multicellular organisms as true superorganisms. Examples are the Termitidae among the termites, and several genera of social Hymenoptera, such as *Pheidole*, *Monomorium*, and *Cardiocondyla* (see **Supplementary Table 1**, for simplicity we only included here species with workers that have completely lost their ovaries).

WHICH FACTORS FACILITATE THE 'PROGRESSION' TOWARD A MAJOR EVOLUTIONARY TRANSITION?

As shown above, step 2, stable group maintenance, is critical to understand the transition toward a MET. Which factors lead to stable group maintenance and may facilitate a MET? For **fraternal transitions**, a major factor for a successful transition seems to be the relatedness among cooperating individuals (e.g., Hamilton, 1964; Korb and Heinze, 2004; Foster et al., 2006; Boomsma, 2009; Abbot et al., 2011). This close evolutionary linkage among cooperating partners can be rephrased as *common ancestry* (relatedness) and *aligned future* (obligate uniparental vertical transmission) for fraternal and **egalitarian transitions**, respectively (Korb, 2010). In addition, all METs are characterised by DOL, and benefits derived from DOL appear to be major drivers for stable group maintenance.

Division of labour emerges spontaneously, when two or more individuals are grouped together (e.g., Fewell and Page, 1999). Hence, DOL plays a pivotal role during the initial steps of group formation (Michod, 2007). When associated with net direct fitness benefits for interacting partners, group maintenance can be selected, along with specialisation of partners for different tasks. The latter results in increased efficiency and fitness (Oster and Wilson, 1978; Michod, 2007; West et al., 2015; West and Cooper, 2016; Cooper and West, 2018) as well as mutual dependency between interacting partners, which are no longer all-rounders (loss of individual totipotency) (McShea, 2002 but also Michod, 2006, 2007; Bourke, 2011; West et al., 2015; Birch, 2017; Cooper and West, 2018). Together with conflict resolution mechanisms, mutual dependency is of fundamental importance for the stability of groups. Benefits associated with increased group sizes can re-enforce these processes, resulting in positive feedback loops with increasing task division and specialisation (Michener, 1964; Karsai and Wenzel, 1998; Michod, 2007; Korb, 2010; Bourke, 2011) and the occurrence of novel emergent properties (for social insects: more efficient communication *via* trail pheromones, construction of mounds) (Korb, 2010; Leonhardt et al., 2016).

Strikingly, all fraternal transitions (note, the multiple independent origins of multicellularity and of superorganismality in social insects) are centred on reproductive DOL. Some parts of the emerging group (multicellular organisms, superorganisms) specialise in reproduction, which are sometimes but not always (e.g., plants, hydra) separated as a germline, while the remaining units perform all the others tasks, such as intake of food, nutrient provisioning, mobility, and/or defence. By outsourcing reproduction versus maintenance to different individuals within a group, reproductive DOL freed individuals (i.e., lower-level units) partially from the corresponding life history constraint. As the "degrees of freedom" increase, life history trade-offs of individuals can be overcome and eventually be reshaped. We, therefore, propose that DOL-associated vanquishing of life history trade-off to be another driver that can lead from group maintenance to group transition, and thus to METs.

THE "VANQUISHING" OF LIFE HISTORY TRADE-OFFS, A SPECIAL "BENEFIT" OF DIVISION OF LABOUR

There are a number of causes for the occurrence of life history trade-offs, some of the most common, non-mutually exclusive ones are: (i) pleiotropic genes, (ii) endocrine mechanisms (e.g., JH, testosterone), (iii) developmental constraints, and/or (iv) limiting resources (i.e., allocation trade-offs) (e.g., Stearns, 1992). Several of these constraints can be overcome either through differential regulation of gene expression between tissues/castes (fraternal transitions) or through "independent evolution" (different gene sets) (egalitarian transitions).

Prominent examples are social insects, which have apparently overcome the omnipresent trade-off between fecundity and longevity (Monroy Kuhn and Korb, 2016 and references therein), that commonly constrains the fitness of solitary organisms. In eusocial insects, the queens (and in termites also kings) are the only individuals reproducing within a colony. At the same time, they can reach lifespans of decades with reproduction increasing longevity, while the non-reproducing workers often live for a few months only (Keller and Genoud, 1997; Keller, 1998; Schrempf et al., 2005; Kramer and Schaible, 2013; Korb and Thorne, 2017). In ants (Kramer and Schaible, 2013) and termites (Korb and Thorne, 2017) longevity of queens increases with social complexity. In termites, the most long-lived females occur in superorganismal species that passed a MET: queens of the fungus-growing *Macrotermes* termites can live for more than 20 years and produce 20,000 eggs per day (Korb and Thorne, 2017). Thus, they are arguably the most fecund terrestrial animals. A mechanistic example of how trade-offs can be overcome are cases of caste-specific gene expression that are associated with gene duplications. Multi-copy genes can be co-opted during evolution for caste-specific functions, thus e.g., vanquishing trade-offs associated with pleiotropic gene functions (Gadagkar, 1997; Korb, 2016). A recent study suggests that this has been the case in termites (Shigenobu et al., 2021). We hypothesise that mechanisms vanquishing life history trade-off, are less studied drivers toward METs, which are most prominent in superorganismal species (Blacher et al., 2017). They might contribute to explain the ecological and evolutionary success of social insects (social Hymenoptera[1]; termites: Kambhampati and Eggleton, 2000; Korb and Thorne, 2017 and references therein). To test the hypothesis that social insects, which have passed a MET are more successful than those that have not, we used species richness as a potential proxy of evolutionary success (for more information, see **Supplementary Table 1**, **Data Sheet 1**). Ant genera with sterile workers have, indeed, an increased species richness compared to those which were non-superorganismal (i.e., no sterile workers, **Figure 1B** – for simplicity of identification, we only used species, in which workers have completely lost their ovaries). For termites, direct comparison between lineages with sterile workers and those without showed a trend for increased species richness in lineages without worker reproduction but this trend disappeared when data were controlled for phylogeny (**Figure 1C**).

DISCUSSION

Our definition of a MET in social insects (sterility of workers, including soldiers, i.e., true neuters) differs from existing definitions (e.g., Helanterä, 2016; Boomsma and Gawne, 2018; Bourke, 2019) and it apparently seems to align with former ones (e.g., Wheeler, 1911; Wells et al., 1929; Buss, 1987; Hölldobler and Wilson, 2009). However, the latter studies, which used worker sterility as a defining hallmark for superorganisms, were not formulated within a MET framework and the authors had different reasons for delimiting superorganisms unlinked to a formal foundation in evolutionary theory. By contrast, we based our criterion on the ultimate explanation that the fitness of these lower-level units is only completely transferred to the higher colony level when there are true neuters. Therefore, our definition offers two major advantages: it is founded in (i) *fundamental first principles of evolution* that (ii) *apply across all METs*. We think these two points are major advantages of our definition.

As, recommended by Herron (2021), our definition is not guided by impressive complex traits of specific taxa, such as the dance language in the honey bee, mound building in some termites or complex societies observed in army ants or fungus-growing ants and termites. Some of these species would qualify as formal *evolutionary* superorganisms, others not. Many other organisms evolved complex traits (e.g., bacterial biofilm). Yet, these organisms would not be considered to have passed a MET. Our criterion is also not guided by specific social insects. Many of the currently available definitions have implicitly social Hymenoptera in mind. For example, "loss of totipotency" as a superorganism/MET criterion does not mean loss of direct fitness in termites. Among the so called "lower termites" there a many species, in which workers lost totipotency as they cannot become winged sexuals but they still commonly reproduce within the natal nest and gain considerable direct fitness (Korb and Hartfelder, 2008; Roisin and Korb, 2011). These species also have morphologically differentiated workers, another criterion sometimes used to define superorganism. Yet, none of these species would be defined as superorganisms in a MET context. Similar arguments would apply to social thrips or aphids (e.g., morphological soldier castes; e.g., Chapman et al., 2008;

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[1] www.antcat.org

Pike and Foster, 2008), which most researcher would not consider as superorganismal.

Our definition could be criticised as Gardner and Grafen (2009) have shown in their model that sterile workers are neither necessary nor sufficient for superorganisms to evolve. Yet, they defined superorganisms "as a group that wields adaptations in its own right" (more like Wheeler, 1911; Wells et al., 1929; Buss, 1987; Hölldobler and Wilson, 2009). This is not the MET concept, we apply here.

An important point in the discussion of distinguishing superorganisms is conflict within colonies. According to our definition there is no conflict over reproduction within monogamous colonies though conflict of the sex ratio may still exist in social Hymenoptera. We argue, this is similar to multicellular organisms, in which conflict over the sex ratio can still occur due to maternal inheritance of, e.g., mitochondria (Burt and Trivers, 2006). One situation under which conflict over reproduction can occur even in species with sterile neuters are polygamous colonies. Polygamy is generally considered to be a derived trait in social insects (e.g., Boomsma, 2009). After a MET, new conflicts can be evolve, similar as after the evolution of individuality in multicellular organisms.

One solution to overcome the conflict argument is the superorganismality approach by Queller and Strassmann (2009) and Strassmann and Queller (2010) to regard associations as a continuum along the two axes of cooperation and conflict (Sherman et al., 1995). While we are much in favour of this concept, it does not allow to delimit METs, which seem to exist in nature. During a transition process – as might be happening in social insects as a whole – it might be difficult to identify specific criteria, which become apparent only later. While our definition can be criticised (e.g., because it excludes some species with very complex sociality), we hope it contributes to clarify what is a MET and how best to define it. We think that METs should be defined using criteria that are based on common ultimate/evolutionary principles that apply across transitions.

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

JK conceived the idea to this manuscript. JK and AB wrote the manuscript. BK edited the manuscript and analysed the data. AB, BK, and JK collected the data. All authors have read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.732907/full#supplementary-material

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OPINION
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What Do We Mean by Multicellularity? The Evolutionary Transitions Framework Provides Answers

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INTRODUCTION

At first glance, the meaning of the word "multicellularity" appears to be unambiguous—it is treated as an "intuitive" concept, something that can be grasped with common sense. On closer inspection, however, it is apparent that there is notable disparity in the recent literature regarding the usage of the term "multicellularity." Whereas, traditionally it was mainly attributed to complex organisms (Grosberg and Strathmann, 2007), more recently it has also been used for simple microbial colonies or biofilms (Hengge, 2020). Accordingly, a unifying definition is lacking—whereas some definitions require cells to display an overall coordination of function (Wolpert and Szathmáry, 2002), have physical contact and strong interactions (Kaiser, 2001), others are simply based on the presence of a group-morphology (Schirrmeister et al., 2013).

We think that it is important to be more precise when using the term multicellularity as, for example, a microbial colony differs in important ways from a multicellular organism like us. This distinction has implications for various areas of inquiry such as "the sociobiology of microbes" and "the evolutionary transition to multicellularity." While these research directions have brought together a highly interdisciplinary community of researchers, adequate descriptions of the marginal or nascent cases of multicellularity remain elusive, despite their identification across the entire range of model organisms, such as algae, protozoans, yeast, and bacteria (Ratcliff et al., 2012; Claessen et al., 2014; Hammerschmidt et al., 2014; van Gestel and Tarnita, 2017; Brunet et al., 2019; Kapsetaki and West, 2019). Lack of continuity has also led to vastly different estimates of the number of instances of multicellular emergence in evolutionary history (Niklas and Newman, 2020). Depending on the definition of multicellularity, it is thought to have evolved from unicellular ancestors on 13–25 independent occasions. When described simply as a cellular aggregation, multicellular organisms are estimated conservatively to have evolved in at least 25 lineages (Grosberg and Strathmann, 2007), making it a "minor major" evolutionary transition. When more stringent criteria are applied, as for example a requirement for sustained cell-to-cell interconnection, communication, and cooperation, multicellularity has evolved multiple times in bacteria (e.g., Actinobacteria, Myxobacteria, and Cyanobacteria; see Bonner, 2000), but only once in the Animalia, three times in the Fungi (chytrids, ascomycetes, and basidiomycetes), and six times among the algae (twice each in the rhodophytes, stramenopiles, and chlorobionta; Niklas and Newman, 2013).

We argue that we need a better understanding about what multicellularity is to meaningfully discuss factors that determine its evolution. We propose that clarity can be achieved with the realization that the various definitions of multicellularity are in fact describing different stages that can occur during the course of its evolution. The major evolutionary transition from single cells to multicellular organisms is not an instantaneous shift, but rather a process with multiple transient stages. As such, "multicellularity" itself is not necessarily a fixed state, but exists as a large range encompassing single cells that are part of multicellular groups, multicellular individuals, and multicellular organisms. We here provide a framework for identifying the various stages of the transition to multicellularity. Importantly, we do not intend to imply that fixed boundaries separate stages of an evolutionary transition from single cells to multicellular organisms. We only demarcate stages here to provide a conceptual link between semantic use and a dynamic evolutionary process.

THE EVOLUTION OF MULTICELLULARITY—A DYNAMIC PROCESS

The transition to multicellularity begins with the evolution of cooperation, where cells unite together and gain an advantage over solitary cells (Stage One; **Figure 1**). The focus of natural selection remains on cells, albeit in a *group-structured* context. Stage Two is the true "transitional stage" of a major evolutionary transition, where the cooperating group also becomes a unit of selection—a "Darwinian individual." Crucially, in order to satisfy the conditions of Darwinian individuality, the groups themselves are subject to a process of reproduction and selection that is more than simply selection among their constituent cells (Godfrey-Smith, 2009). A high degree of functional organization is an adaptation of groups, *resulting from* selection operating at the higher (group) level (Okasha, 2006). Therefore, complex adaptations of groups accumulate during the third stage of an evolutionary transition. Eventually, group adaptations lead to such integration of the cells comprising the group that they can no longer exist independently, and now only survive and replicate as components of the multicellular group—the "organism" (Stage Three). In contrast to the view of Bourke (2011), who proposes that only complex multicellular organisms possess individuality, in our view individuality occurs at an earlier phase of the transition (Stage Two).

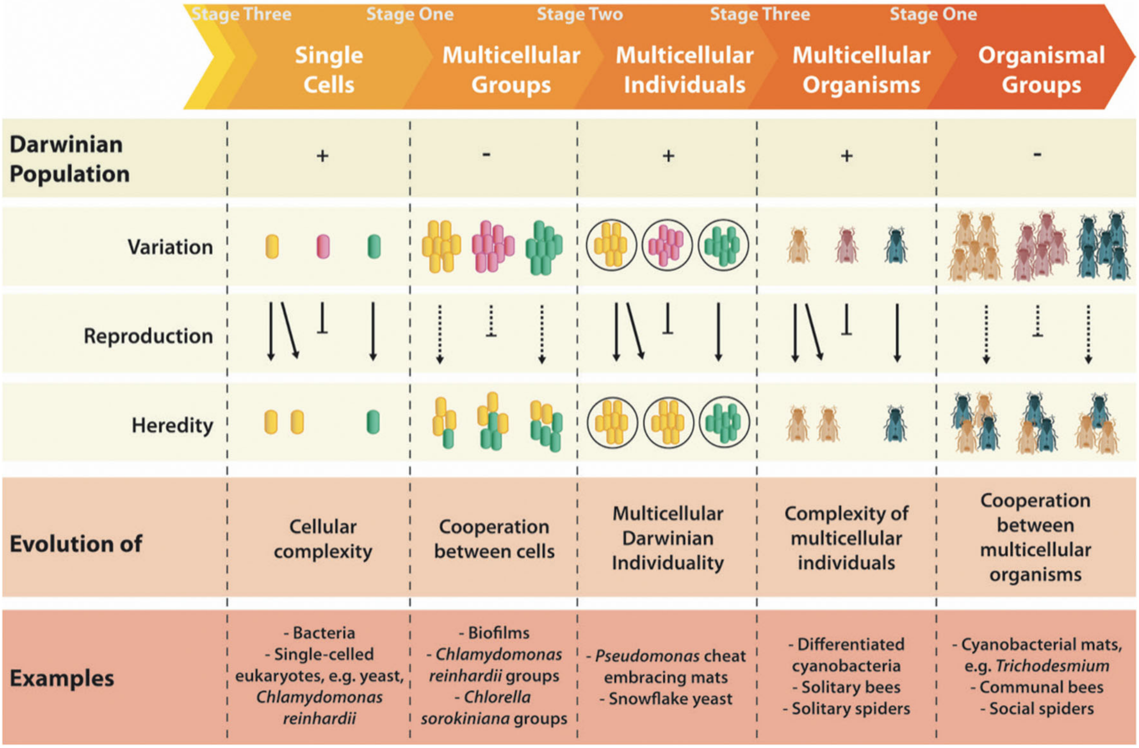

FIGURE 1 | Multicellular entities are defined as Multicellular Groups, Multicellular Individuals, or Multicellular Organisms, reflecting the stages of the evolutionary transition from single cells to multicellularity imbedded within the hierarchical structure of life. "Darwinian populations" are populations of "Darwinian individuals" or "units of selection," which are entities that can evolve by natural selection by virtue of possessing these essential characteristics: variation between entities within the population, which is heritable and causally associated with their differential reproduction (Lewontin, 1970). The (+) symbol signifies that, for example, single cells are units of selection in a Darwinian population (of cells). While multicellular groups may display a marginal kind of reproduction by virtue of differential growth (i.e., reproduction of cells), they do not meet other criteria for Darwinian individuality, such as (group) heredity, and therefore the (–) symbol indicates that multicellular groups are not members of a Darwinian population (of groups). Such criteria are the subject of much debate, and include both intrinsic features and ecological requirements (see Stage Two). Colors of different entities represent genetic differences; solid arrows and bars represent differential reproduction, while dotted arrows and bars represent differential growth of groups.

STAGE ONE—EVOLUTION OF MULTICELLULAR GROUPS

The evolution of cooperation encapsulates Stage One of the evolution of multicellularity. A cooperative behavior is generally described as a costly investment in resources that benefits an individual (the recipient) other than the actor (Chase, 1980), regardless of whether the recipient adopts the same behavioral strategy. Cooperative interactions are central to an evolutionary transition because the necessary fitness cost associated with cooperation is offset by a group-level benefit.

During the transition to multicellularity, cooperation between cells resulted from the advantages gained by adhering to each other. This occurred through two mechanisms: clonality and aggregation (Grosberg and Strathmann, 2007; Tarnita et al., 2013). During a transition to clonal multicellularity, cells fail to adequately separate after cell division and ergo remain attached. Consequently, clonal forms of multicellularity, such as plants and animals (Stage Three), developed from a small number of cells (Stage One/Two)—an evolutionary "bottleneck." The aggregative mode of multicellularity usually results from motile single cells (Stage One/Two) clustering together to form fruiting bodies for sporulation and dispersal, often in response to environmental starvation (Gross, 1994). Aggregative forms of multicellularity have arisen independently in eubacteria, several cellular slime molds, and in ciliates (Bonner, 1998). While aggregative forms of multicellularity are numerous and widespread, particularly in terrestrial environments, clonal multicellularity has led to greater diversity and complexity (Fisher et al., 2013).

The challenge for understanding the evolution of cooperation is explaining how cooperation generates a benefit (Calcott, 2011). Multicellular cooperation in many lineages may have originally obtained the advantage of increased size afforded by the ever-present open niche at the top of the size scale (Bonner, 1998, 2000). Proposed advantages of increased size are that larger assemblages of cells avoid predation by filter feeders or that increased size enhances feeding efficiency (Dworkin, 1972; Bell, 1985; Bonner, 1998; Boraas et al., 1998; Pfeiffer et al., 2001; Alegado et al., 2012; Koschwanez et al., 2013; Herron et al., 2019; Kapsetaki and West, 2019). Other advantages of cellular cooperation include benefits associated with both fixed surface attachment and enhanced dispersal. Single cells located in an ideal position for growth may be swept away by currents or wind, whereas an increased ability to adhere to surfaces by cell clusters might be selectively advantageous (Gross, 1994; Bonner, 1998). Tradeoffs between two incompatible processes that cannot be performed in one cell at the same time have also been proposed as important drivers of multicellular cooperation. Examples of such tradeoffs include motility and mitosis in metazoans (Margulis, 1981; Buss, 1987; King, 2004), reproduction and motility in the volvocene green algae (Koufopanou, 1994), and N_2 and CO_2 fixation in cyanobacteria (Rossetti et al., 2010; Herrero et al., 2016; Hammerschmidt et al., 2021).

STAGE TWO—EVOLUTION OF MULTICELLULAR INDIVIDUALS

Stage Two is the true "transitional" phase of a major evolutionary transition because during this stage, natural selection operates between groups, rendering them "Darwinian individuals." In order to be a "unit of selection" (Lewontin, 1970), a group itself must become capable of a form of reproduction that allows selection to operate on the variation between groups, *over and above* selection already occurring between cells (**Figure 1**). The particular question of relevance to major evolutionary transitions is the puzzle of group reproducers (Godfrey-Smith, 2009)—reproducing units comprised of particles which themselves have the capacity to reproduce. Multicellular groups, for example biofilms or *Chlamydomonas reinhardtii* groups (Herron et al., 2019), multicellular individuals, such as snowflake yeast (Ratcliff et al., 2012) or *Pseudomonas* cheat embracing mats (Hammerschmidt et al., 2014), and multicellular organisms (filamentous cyanobacteria, solitary bees) are all examples of group reproducers. The difficulty is to identify which are cases of reproduction of groups, and which are cases of growth of groups resulting from reproduction and structural organization of their particles (see **Figure 1** for details).

This challenge is related to the problem of explaining how groups acquired a fundamental requirement for reproduction—a life cycle. The particular mode by which the earliest multicellular groups reproduce, for example through a dedicated (germ) cell or by fragmentation, has implications for their ability to transition in individuality and participate in natural selection (Ratcliff et al., 2012; Hammerschmidt et al., 2014). Furthermore, during this transitional phase, ecological conditions are of critical importance (Pichugin et al., 2019; Staps et al., 2019), such as structured environments that maintain the discreteness of groups, and crucially, their reproductive cells (Rose et al., 2020). Such conditions provide the ecological scaffold for selection to act on less-integrated groups until they complete the transition to "multicellular individuals" (Black et al., 2020). The challenge of identifying criteria for Darwinian individuality has been the subject of much recent discussion. Our aim here is not to review the mechanisms put forward to explain the transition to Darwinian individuality (e.g., Michod, 2005; Godfrey-Smith, 2009; Bourke, 2011; Hammerschmidt et al., 2014; Black et al., 2020; Rose et al., 2020; Bourrat et al., 2021), but rather to remove linguistic ambiguities that may impede fruitful debate.

STAGE THREE—EVOLUTION OF MULTICELLULAR ORGANISMS

After a multicellular group becomes a Darwinian individual, it is possible for natural selection to operate on traits that enhance the fitness of the group as a collective unit. The accumulation of such traits leads to the evolution of progressively higher complexity. Hence, the term "complexity" does not refer to a specific state reached by a multicellular organism, but it is a relative term used to describe a wide spectrum of collective functions.

Multicellular complexity is often represented by the number of different cell types coexisting in the collective, although epigenetic control of this cellular differentiation is clearly an important innovation resulting from group-level selection (Buss, 1987; Arnellos et al., 2013). Epigenetic regulation of development itself evolves as increasingly more complex genetic networks. The accumulation of group adaptations may eventually lead to such a degree of integration of parts that the cells no longer exist independently—their survival and reproduction depends entirely on the survival of the group. We suggest that this loss of lower level autonomy be the defining feature of the term "organism," ultimately rendering an organism indivisible. In the level above multicellular organisms, eusocial insect colonies are sometimes referred to as "superorganisms" when the lower level units no longer exist autonomously and instead subsist as sterile workers. This has also been shown to involve an increase in complexity of gene networks (Kapheim et al., 2015).

The evolution of developmental regulation is mechanistically unproblematic because the genetic machinery for coordination of differentiated cell types existed in primitive "multicellular" prokaryotes and close eukaryotic unicellular relatives of metazoans (Gombar et al., 2014; Glöckner et al., 2016; Sebé-Pedrós et al., 2016; Brunet and King, 2017). It is therefore surmised that few mutational steps should be required in a regulatory pathway to produce additional cellular differentiation. Indeed, thousands of differences in gene expression between cell types in multicellular organisms are often controlled by a small set of regulatory proteins. This is supported by the fact that the presence of many genes underlying multicellular development and function has been inferred in the unicellular ancestors of metazoans, algae, and fungi, providing strong indications that regulatory changes indeed led to the co-option of the ancestral genes (Hanschen et al., 2016; Sebé-Pedrós et al., 2016; Kiss et al., 2019). Nevertheless, important metazoan developmental gene families, notably the Hox genes, are not present in unicellular ancestors (Ruiz-Trillo et al., 2007), indicating that these gene regulatory pathways evolved later as a consequence of multicellular individuality.

CONCLUSION

The transition to multicellularity is of seminal biological significance as it led to the vast biological complexity and diversity we see on our planet today. Reconstructing the stages that occurred during the process of evolutionary transitions that took place in the distant past is a major challenge (Maynard Smith and Szathmáry, 1995). While most research has focused on theoretical and philosophical aspects of these events, several recent developments and novel techniques have transformed this research area and brought together a highly interdisciplinary community of researchers who are rapidly advancing the field. One novel approach has been the utilization of unicellular model organisms, such as yeast, algae, protozoans, and bacteria in experimental evolution studies to mimic the evolution of early stages of the transition to multicellularity (Ratcliff et al., 2012; Claessen et al., 2014; Hammerschmidt et al., 2014; van Gestel and Tarnita, 2017; Brunet et al., 2019; Kapsetaki and West, 2019).

This new research direction has already contributed many exciting results that feed back into theory. However, these studies have also led to confusion regarding the definition of the term "multicellular," because they focus on marginal or nascent cases of multicellularity. In addition, the utility of the various definitions of multicellularity remains vague for extant organisms (Kaiser, 2001; Wolpert and Szathmáry, 2002; Grosberg and Strathmann, 2007; Schirrmeister et al., 2013; Hengge, 2020). We advocate that clarity can be achieved by considering the diverse use of the term "multicellularity" as sequential stages of a dynamic evolutionary process, from multicellular groups, to multicellular individuals, and finally to multicellular organisms. Semantic continuity among researchers will lead to more productive communication between evolutionary biologists and ecologists, microbiologists, philosophers, physicists and theoreticians, further advancing this exciting field.

AUTHOR CONTRIBUTIONS

Both authors contributed to the article and approved the submitted version.

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Major Evolutionary Transitions and the Roles of Facilitation and Information in Ecosystem Transformations

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A small number of extraordinary "Major Evolutionary Transitions" (METs) have attracted attention among biologists. They comprise novel forms of individuality and information, and are defined in relation to organismal complexity, irrespective of broader ecosystem-level effects. This divorce between evolutionary and ecological consequences qualifies unicellular eukaryotes, for example, as a MET although they alone failed to significantly alter ecosystems. Additionally, this definition excludes revolutionary innovations not fitting into either MET type (e.g., photosynthesis). We recombine evolution with ecology to explore how and why entire ecosystems were newly created or radically altered – as Major System Transitions (MSTs). In doing so, we highlight important morphological adaptations that spread through populations because of their immediate, direct-fitness advantages for individuals. These are Major Competitive Transitions, or MCTs. We argue that often multiple METs and MCTs must be present to produce MSTs. For example, sexually-reproducing, multicellular eukaryotes (METs) with anisogamy and exoskeletons (MCTs) significantly altered ecosystems during the Cambrian. Therefore, we introduce the concepts of Facilitating Evolutionary Transitions (FETs) and Catalysts as key events or agents that are insufficient themselves to set a MST into motion, but are essential parts of synergies that do. We further elucidate the role of information in MSTs as transitions across five levels: (I) Encoded; (II) Epigenomic; (III) Learned; (IV) Inscribed; and (V) Dark Information. The latter is 'authored' by abiotic entities rather than biological organisms. Level IV has arguably allowed humans to produce a MST, and V perhaps makes us a FET for a future transition that melds biotic and abiotic life into one entity. Understanding the interactive processes involved in past major transitions will illuminate both current events and the surprising possibilities that abiotically-created information may produce.

Keywords: adaptation, innovation, facilitation, information, ecosystem

INTRODUCTION

All adaptations, by definition, increase the fitness of organisms in their environments, but few merit special consideration as extraordinary (Stebbins, 1969; Bonner, 1974; Buss, 1987; Queller, 2000; Bourke, 2011a; Calcott and Sterelny, 2011; Szathmáry, 2015; West et al., 2015). Maynard Smith and Szathmáry in an influential book (1995) and paper (Szathmáry and Maynard Smith, 1995) listed eight as Major Evolutionary Transitions (METs) (**Table 1**). Bourke (2011a) and Szathmáry (2015) amended this list, with the latter explicitly stating that the MET concept is not in relation to "ecosystem complexity, but [to] the complexity of the players . . . acting in the ecological theater" (p. 10,104). Overall, across these contrasting lists (**Table 1**) there are two broad classes of adaptations that qualify as gains in "organismal complexity" and constitute METs. One is 'Fusions,' where independently reproducing entities combine into higher, integrated levels of obligate reproductive cooperation (Buss, 1987; Michod, 1996; Szathmáry, 2015; West et al., 2015). Factors that could both favor and stabilize formation of Fusions include selective advantages of division of labor and mutual dependence (West et al., 2015), the maximization of inclusive fitness (Bourke, 2011a; West et al., 2015), and the ability to punish cheaters (Ågren et al., 2019). The other class is 'Information Leaps': novel forms of information storage or transmittal across individuals, ranging from genes to symbolic writing (Jablonka and Lamb, 2006).

TABLE 1 | Proposed major evolutionary transitions.

Maynard Smith and Szathmáry, 1995	Bourke, 2011a	Szathmáry, 2015
Independently replicating molecules to populations in protocell compartments (IL)	Independently replicating molecules to cells enclosing DNA genomes (F and IL)	Independently replicating molecules to cells enclosing genomes (F and IL)
Independent replicators to chromosomes (F)	Combined into the above	Combined into the above
RNA to DNA as genetic code and information system (IL)	Combined into the above	RNA to DNA as genetic code and information system (IL)
Prokaryotes to eukaryotes (F)	Prokaryotes to eukaryotes (F)	Prokaryotes to eukaryotes (F and IL)
Asexual to meiotic sexual reproduction (IL)	Asexual to meiotic sexual reproduction (IL)	Combined into the above
		Acquisition of plastids (e.g., chloroplasts) (F)
Single celled protists to complex multicellularity in plants, animals, and fungi (F)	Single celled protists to complex multicellularity in plants, animals, and fungi (F)	Single celled protists to complex multicellularity in plants, animals, and fungi (F)
Solitary individuals to eusocial groups with non-reproductive castes (F)	Solitary individuals to eusocial groups with non-reproductive castes (F)	Solitary individuals to eusocial groups with non-reproductive castes (F)
Primate societies to human societies with advanced language (IL)	Not considered a MET	Primate societies to human societies with advanced language (IL)
	Interspecific mutualism (F)	

Transitions can be fusions (F) that produce higher-level individuals, or information leaps (IL) that innovate new ways of storing information and transmitting it across individuals.

Limiting METs to Fusions or Information Leaps, regardless of ecosystem-level impacts (Szathmáry, 2015), excludes a number of other possibly "major" events. Although such designations can vary subjectively, considering a wider array of events can illuminate intriguing commonalities, dependencies, and unanswered questions that contribute to understanding the history of life on Earth. In this paper, we: (1) Introduce a more inclusive set of terminology to improve future discourse on major transitions (**Figure 1**), and (2) explore how major ecosystem transitions arise within broad frameworks that can include multiple Fusions and Information Leaps, morphological innovations, catalytic actors and events, and variation in the selective processes involved.

Major Events in the History of Life

We retain Szathmáry's (2015) definition of Major Evolutionary Transitions (METs) as being Fusions and Information Leaps, and introduce the term Major System Transitions (MSTs) to describe large-scale ecosystem transformations that appear irreversible. We note that even in the most extreme mass extinctions, while many or most species went extinct, we know of no case in which an entire large-scale ecosystem disappeared or reverted to an earlier state (e.g., the Permian extinction did not reset the world back to the Ediacaran).

Additionally, we consider morphological adaptations that confer significant direct-fitness advantages. For instance, many regard the water-to-land (Knoll and Bambach, 2000; Qiu, 2008; Van Etten and Bhattacharya, 2020) and land-to-water transitions (Aubret et al., 2007; Schwab et al., 2020) as major evolutionary events, although these did not produce novel forms of individuality or information. We define such remarkable morphological adaptations as Major Competitive Transitions (MCTs), while acknowledging the definition's subjective nature. Here, "competitive" implies more than a slight advantage in terms of survival and/or reproduction. It implies great fitness benefits due to, for example, creating a new niche (e.g., the evolution of flight), or dominating an existing niche in a novel way (e.g., vascular tissue in land plants). Clearly, there are many examples of innovative morphologies that could potentially qualify as MCTs and lead to MSTs.

Whereas MSTs happen to ecosystems, METs and MCTs happen to species. MET and MCT categories are not mutually exclusive. For example, the evolution of larger brains and greater cognitive abilities (a MCT) can simultaneously lead to an Information Leap of complex human spoken language (a MET). However, not every MET, MCT or combination of the two necessarily immediately leads to a MST. Consider the evolution of eukaryotes from prokaryotes.

Eukaryotic single-celled organisms appear in the fossil record perhaps by 1.6 BYA (Knoll et al., 2006). Yet for a "boring billion" years of evolutionary history, they remain minor components in bacterial-dominated ecosystems before explosively radiating as large, multicellular species in an Ediacaran and Cambrian MST. Eukaryotes are obviously essential for this MST, as all animals, plants and fungi are eukaryotes. However, the initial

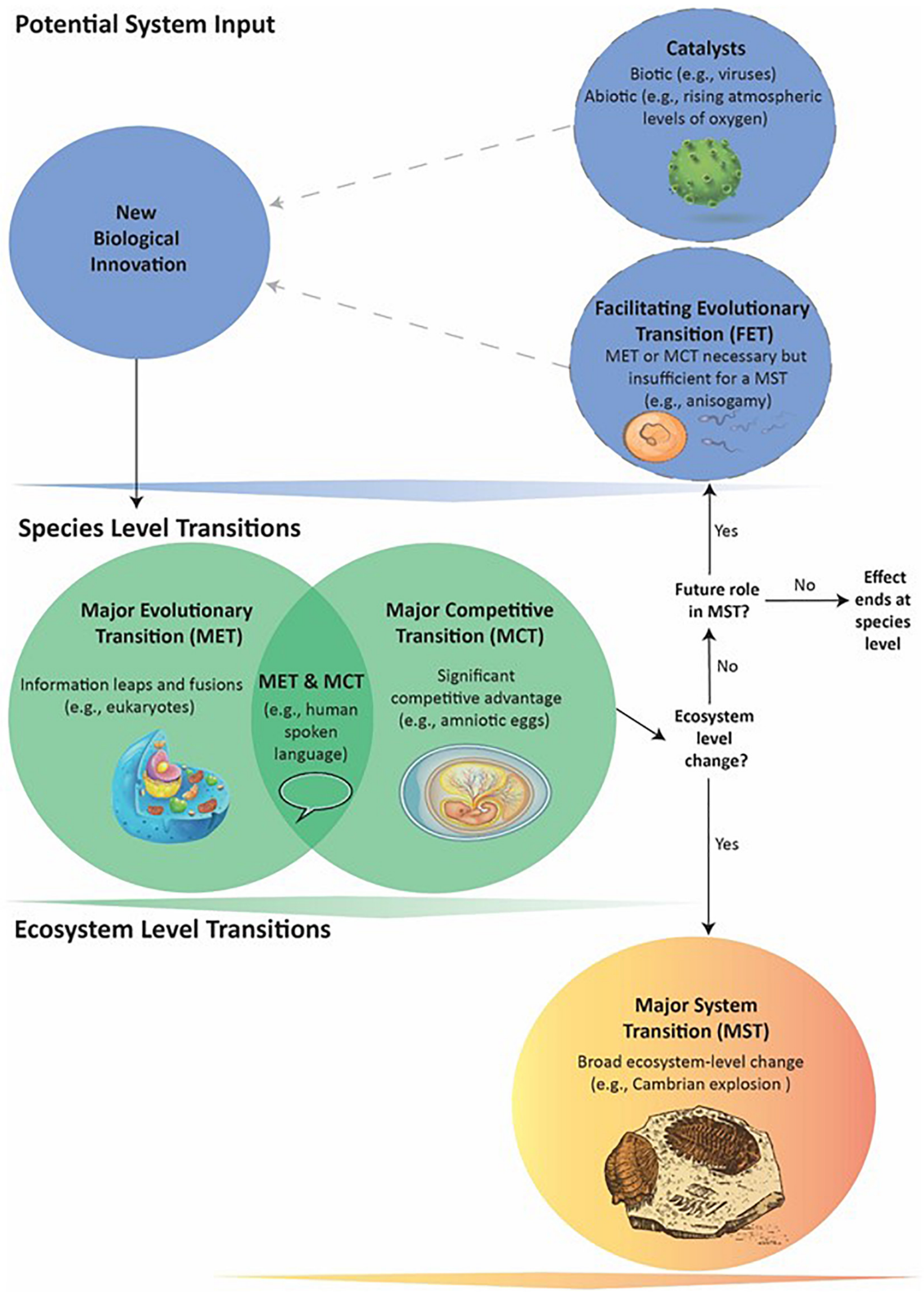

FIGURE 1 | General schematic of how a series of events can lead to a Major System Transition (MST). Biological innovations are system inputs that may result in Major Evolutionary or Competitive Transitions (METs and MCTs – see text for definitions). An innovation can become a MET or MCT: (1) through its own direct effects; (2) with requiring a previous MET or MCT that acts as a Facilitating Evolutionary Transition (FET); or (3) as catalyzed by biotic agents or abiotic events. The dotted lines indicate FETs and catalysts may or may not be present. An innovation producing a MET, MCT, or both occurs at the Species level and its downstream effects can directly lead to MSTs: large-scale, ecosystem-level transformations affecting many species. Not all individual METs and MCTs, however, appear capable of causing MSTs. Nevertheless, they can still potentially act as FETs that in combination with later METs or MCTs do lead to a MST. In such cases, the evolutionary process cycles through more than one Species-level transition before the MST.

appearance of eukaryotic cells seems insufficient for a MST. Thus, "eukaryote" is both a MET (*sensu* Maynard Smith and Szathmáry) and a Facilitating Evolutionary Transition, or FET, for the later MST. We define a FET as either a MET or a MCT that is absolutely necessary, yet insufficient alone, to set into motion a cascade of events that result in a MST. In other words, species that have undergone a FET require additional events (e.g., other MCTs, METs, or external "catalysts") before the combination of events alters environments enough to transform or create entire ecosystems (**Figure 1**). A catalyst can be an abiotic event such as rising levels of free oxygen in the atmosphere making possible the energetic lifestyles of multicellular organisms (Och and Shields-Zhou, 2012; Lyons et al., 2014). Catalysts can also be biotic actors such as viruses and bacteria that through coevolutionary arms races and horizontal gene transfers help drive certain METs, MCTs, and MSTs (**Figure 1**).

Levels of Information and Selective Processes

Information – defined here as data in a repository that organisms may use to respond to problems they face or to manipulate their environments – can play a fundamental role in METs that produce MSTs. Building on the framework of Jablonka and Lamb (2006), we categorize information into five distinct levels (**Table 2**). Level I information is encoded in genomes. The simplest currently existing units using only Level I information are viruses of less than 10 genes. Level II information is epigenetic modification of gene expression. As such, Level II information can alter how organisms use Level I information throughout their lifetimes due to interactions with environments. Beyond Level II, bounds on total information content are progressively lifted. At Level III, information is learned and stored in neural cell repositories, and potentially interchangeable across entire populations. Level IV information is stored outside of organisms in the physical environment, either as biological icons (e.g., scent marks or pheromone trails) or instructional (symbolically inscribed). Level IV information reaches its apogee with the advent of symbolic representation of human language into quantitatively unlimited written and electronic formats. Finally, we propose a new and nascent level of information. Level V is dark information created by complex computer algorithms rather than biological beings. It is 'dark' in the sense that informational outcomes cannot be replicated without the technology. For example, the only test of facial recognition by machine learning is the accuracy of output – the generating process may be impenetrable to human understanding.

We note that the 'body of knowledge' contained at any of the five levels across entire populations can rarely, if ever, be wholly available to or expressed in any single individual. Populations will be genomically more diverse, learn a larger variety of things, and write and read more books than any individual possibly can. Furthermore, although Information Leaps create potential for new and larger pools of information, the initial benefits of such information may be small. For example, the first organisms with any Level III learning ability undoubtedly had a meager capacity for 'knowing' more than their antecedents with only Levels I and II. It likely took millennia for the ability to acquire and use such information to confer significant selective advantages.

Finally, the important evolutionary processes may differ across major transitions. For instance, the evolution of some METs

TABLE 2 | The five levels of information.

Information	Description	Transmission
(I) Encoded	Information format is in DNA (or RNA in some viruses) sequences to guide growth and development either as genes or regulatory series that control expression. This level of information is both an absolute requirement and defines the first biological 'living' replicators.	Information is transmitted vertically via cell replication, although occasionally horizontal transmission across organisms occurs via viruses and plasmids. Sexual reproduction shuffles encoded information within populations and therefore increases total interchangeable information available at the population level.
(II) Epigenetic	Physical modifications of genomes (e.g., methylation) occur during organism lifetimes and translate into changes in physiology, morphology and behavior.	Information passes vertically across cell generations through imprinted genes, and likely first appeared when replicators aggregated into chromosomes.
(III) Learned	Information is gathered during an organism's lifetime and is stored in specialized cells (possible only in complex multicellular species). The available information for organisms can be a quantum leap in amount and outlive any single individual.	Information, as cultural traits, is communicated vertically (parent to offspring), horizontally (peer to peer), or obliquely (across generations through non-parental individuals or institutions). Individuals do not need to directly experience events to gain access to information.
(IV) Inscribed (Iconic)	An icon is a physical inscription such as a scent mark left by a wolf pack to mark its territory, or the trail pheromone of an ant colony recruiting to a food source.	Icons can pass simple bits of information between individuals indirectly without those individuals ever meeting, but does require sensory capabilities.
(Instructional)	Information is transformed into physical, symbolic formats that have potentially boundless storage capacity. Instructional information can far exceed the combined encoded, epigenetic, learned and iconic information previously available to any single individual.	Information is potentially immortal as it does not necessarily depend on the survival of any single individual or group. Across the tree of life, only humans are known to have ever extensively created and used instructional information.
(V) Dark	Information produced by abiotic computer programs, so complicated in execution that no biological organisms can similarly replicate or derive it. Examples of dark information generators are: internet search engines; global climate models; bioinformatic analyses of vast genetic data sets; neural network simulations; and genetic algorithm evolutionary models.	The potential reach of this information may exceed that of the species that creates it, to the extent that it may become a new 'living species' in and of itself (i.e., artificial intelligence: AI). Concepts of reproduction, heredity, species and fitness will need to be redefined.

might require kin selection or multilevel selection (Bourke, 2011a; Szathmáry, 2015; West et al., 2015), whereas MCTs spread through populations because of their direct-fitness advantages. In addition, major transitions can themselves create novel selective processes. For example, sexual reproduction through meiosis (a MET) was likely initially isogamous (Hanschen et al., 2018). Not until anisogamy (a MCT) evolved could sexual selection become a major evolutionary force. In essence, some biological innovations create a morphology-to-process feedback loop. Thus, anisogamy creates different selective pressures for individuals that produce eggs or sperm. Once sexual selection exists, it is not limited to affecting gametes, but also affects other morphologies and behaviors. It may be this newly-altered fitness landscape that enables a future MST rather than any intrinsic ecological advantage of different sized gametes.

In this paper, we reconsider the major events in the history of life on Earth, from the first cells to the recent technological developments of human societies. We focus primarily on which METs identified by Maynard Smith and Szathmáry (1995) have produced MSTs, either directly or in combination with MCTs and catalysts. In reexamining these major transitions, we also highlight the importance of information for both the METs and the resulting MSTs, and speculate upon the role that Level V dark information may play in a future major transition.

INDEPENDENT REPLICATORS TO CELLS WITH CHROMOSOMES

The first MET is the transition of self-replicating molecules into compartmentalized cells with chromosomes (**Table 1**). These higher-level units arguably constitute the first living organisms – entities capable of responding to stimuli, acquiring resources, metabolizing, maintaining homeostasis, and replicating. To the degree they gained a competitive advantage relative to simple replicators, this MET might also be a MCT. Because it produced Earth's first ecosystem, it is a MST. Although many questions about the origin of cells remain unanswered, a variety of hypothesized scenarios may have led to this transition.

Consider a population of short, non-cooperative, independently replicating molecules. Some replicators drive others to extinction, reducing the overall amount of available information (Szathmáry and Maynard Smith, 1995). Linking these replicators into chromosomes reduces competition, but increasingly longer molecules become susceptible to major mutations that disrupt replication (Eigen, 1971). This creates a circularity – Eigen's paradox – where larger genomes require enzymes that increase replication fidelity, but substantially larger genomes are needed to code for such enzymes. Two models are proposed to overcome Eigen's paradox and thus explain how this MET arose: the hypercycle and stochastic corrector (SC).

In the hypercycle model, simple replicators facilitate their own as well as others' replication in a catalytic loop (Eigen, 1971; Eigen and Schuster, 1979; Zintzaras et al., 2002; Kun et al., 2005; Szathmáry, 2006; Saakian et al., 2011; Boza et al., 2014). 'Parasitic' molecules that do not catalyze the replication of others can invade, however, if hypercycles catalyze the parasite's replication (Bresch et al., 1980; Hogeweg and Takeuchi, 2003). Compartmentalization into vesicle-bound "chemotons" is proposed to limit such parasitism (Eigen et al., 1981; Michod, 1983; Szathmáry and Demeter, 1987; Zintzaras et al., 2002; Gánti, 2003). In the SC model, replicators are bound in protocells and their replication is catalyzed by non-specific replicase molecules (Szathmáry and Demeter, 1987; Szathmáry, 1989; Grey et al., 1995; Zintzaras et al., 2002). Certain compositions of replicators are optimal for overall cell growth and excessive competition between them leads to failure of the entire protocell (Grey et al., 1995).

In both hypercycle and SC models, selection against cheating may occur through aggregation of the interacting elements into bounded and competing groups. This would be the first instance of group-level selection. Alternatively, catalyzation of others' replication may be the first manifestation of kin selection if the interacting replicators are morphologically identical or similar (Levin and West, 2017). Kin and group selection frameworks, however, converge when compartmentalization in protocells enables assortment of these identical or similar individuals (Szathmáry and Demeter, 1987; Hogeweg and Takeuchi, 2003; Levin and West, 2017).

With a self-sustaining population of protocells, novel machinery can evolve. Simulations in an SC context find that division of labor arises where RNA strands diverge into biased function for either information storage or enzymatic activity (Boza et al., 2014). Similarly, it seems plausible that some replicators in a hypercycle evolve as the system's metabolism, creating a division of labor that leaves only one type of replicator as the essential heritable repository for Level I information.

Chromosomes

The next question about the first living organisms pertains to information organization and amalgamation of separate, replicating genes into a single unit – a chromosome. The linking of genes into "proto-chromosomes" may have occurred in primordial RNAs before transitions to DNA and translation (Weiner and Maizels, 1987; Maynard Smith and Szathmáry, 1993). Advantages of simultaneously replicating chromosomes may include: (1) decreased reproductive competition among individual segments; (2) increased likelihood that cell division provides entire genomic complements to both daughter cells; and (3) increased capacity to reduce mutation loads (Maynard Smith and Szathmáry, 1993; Santos, 1998). Even if replication of two linked genes takes twice as long as for one gene, models find that linkages increase in frequency under some conditions (Maynard Smith and Szathmáry, 1993). However, the exact mechanism by which chromosomes initially formed and conditions that favored their evolution remains unknown.

Overall, the transition from simple replicators to cells with chromosomes is a Fusion MET and a MST that produced the first ecosystem of living organisms. Details of how this MST occurred are unclear, with possible facilitating adaptations and catalysts yet to be discovered.

TRANSITION TO DNA, GENOME EXPANSION, AND EPIGENETIC MODIFICATIONS

The amount of information encoded in Levels I and II depends on which nucleic acid (RNA or DNA) constructs the genome, the genome's size, and the degree to which genetic sequences produce different states of expression (e.g., epigenetic modification adds another layer of information 'above' the fixed nucleotide sequence).

RNA Versus DNA

Molecular RNA is thought to be closely associated with the origin of life because it has the potential to both store information and simultaneously retain enzymatic activity (Bernhardt, 2012). The information storage capability, but not the self-replicating ability, is observed in the current-day RNA viruses. However, such genomes are highly mutable and therefore RNA viruses succeed within hosts as evolving mutant swarms rather than stable clonal lineages (Ebrahimi and Nonacs, 2021). RNA genomes especially risk crossing into 'error catastrophe' where mutations accumulate at rates that make entire populations non-viable (Summers and Litwin, 2006; Manrubia et al., 2010). Thus, relying on RNA for information storage likely limits organisms to viral-size genomes and severely restricts the potential for future morphological and behavioral adaptations.

The transition to DNA may have been catalyzed by coevolutionary arms races between the ancestors of cells and viruses (Durzyńska and Goździcka-Józefiak, 2015). The first functional cells must have had two salient characteristics: (1) higher concentrations of needed building materials for life than found in the outside environment, making them rich targets, and (2) poor defenses, given that no cellular predators yet existed. Undefended rich targets would create selection for alternative life histories (Koonin, 2016). One life form – the protocells – hunts for resources to grow and divide. The second form – the first viruses – hunts protocells in order to invade and usurp their genomes. Future major transitions may have been impossible without viral catalysts (**Figure 1**) creating a predator-prey world.

With a transformed cellular world of parasite/predator and host/prey, host defenses would evolve by natural selection. One defense may be the inactivation or destruction of invading RNA genomes (Forterre, 2002). To evade such destruction, viruses may have evolved to retrotranscribe RNA into DNA. This would not only increase their chances of taking control of cells, but also confer a selective advantage because DNA is the more stable information repository (Forterre and Prangishvili, 2013). Alternatively, DNA could have been repurposed in hosts from a structural role in order to segregate information from viral invaders (Wolf and Koonin, 2007; Koonin, 2016). Once DNA began storing information, however, its spread throughout protocells may have occurred rapidly due to the superior molecular stability.

Therefore, the switch from RNA to DNA is an Information Leap MET that substantially increases the capacity for Levels I and II information storage (Maynard Smith and Szathmáry, 1995). Whether the initial advantages of DNA were great enough to constitute a MCT, and the extent to which this transition alone transformed ecosystems, may never be known. If not a MST, however, this transition is certainly a FET that enabled the entire subsequent history of life and future MSTs.

Genome Expansion

There are huge variations in genome size and cellular complexity across the three domains of life. Eubacterial and archaeal genomes are generally 0.5–7.6 megabases, while eukaryote genomes are at least 100 megabases (Lynch and Conery, 2003). To the degree that organismal complexity is limited by genome size (Bird, 1995), it is important to understand the processes by which eukaryotic genomes grew in size and information content. Initially, Level I information in eukaryotes was likely similar to that of prokaryotes, and the increase to present-day Level I information required various mechanisms that enlarge genomes. It seems likely that increasing Level I information contributed as a MCT that allowed eukaryotes to remain competitive in prokaryote dominated ecosystems.

Genome duplication immediately increases genome size. Duplications can be autopolyploid (within species, with multiple sets of the same chromosomes) or allopolyploid (e.g., the doubling of a hybrid genome to restore the homologous chromosome state). Over evolutionary time, initially redundant genes can diverge to produce novel functions. Several such genome expansion events appear to have occurred in eukaryotes (Van de Peer et al., 2017).

Horizontal transfer of DNA across species' genomes can add one or more genes at a time. For example, lysogenic viruses enter nuclear genomes and may either never leave or leave behind DNA transported from other species (Hernández and Podbilewicz, 2017; Valansi et al., 2017). Over time imported elements can be co-opted within host genomes to produce novel functions (Werren, 2011), enhance gene regulation (Diehl et al., 2020), or modify horizontally imported genes for analogous functions. Indeed, key processes involved in sexual reproduction appear to be made possible only through imported viral genes (Hernández and Podbilewicz, 2017).

Additionally, endosymbionts (organisms that live within the cells of others), such as the bacterial ancestor to the mitochondria or the parasite *Wolbachia*, often exhibit a biased flow of genetic material into nuclear chromosomes. Even if initial DNA transfers are random in direction, the consequences of meiotic cell division result in nuclei becoming the absorbing collectors of genetic information (Nonacs and Tolley, 2014).

Epigenetics

Epigenetic modifications to DNA (Level II information) occur in all three domains of life (Casadesús and Low, 2006; Willbanks et al., 2016). Furthermore, mRNAs can also be epigenetically modified, suggesting this process could have been present in the RNA world (Garber, 2019). Eukaryotes, however, exhibit more sophisticated epigenetic regulation of gene expression than prokaryotes (Willbanks et al., 2016). This regulation likely preceded the evolution of multicellularity as single-celled eukaryotic species change their epigenetic states in fluctuating environments without altering genome sequences (Kundu et al., 2007; Payne et al., 2019; Bheda et al., 2020).

Level II epigenetic information creates alternative readings of Level I information, and empowers Lamarckian inheritance to transmit acquired characteristics across several generations of cell division (Jablonka et al., 1998; Jablonka and Lamb, 2006). In multicellular eukaryotes, such epigenetic modification can aid in differentiating dividing clonal cell lineages into specialized functions (Juliandi et al., 2010; Wang et al., 2021). Whether or not the appearance of epigenetic information significantly altered ecosystems (a MST) or provided a large fitness advantage (a MCT) is unknown. However, the evolution of epigenetics clearly qualifies as an Information Leap MET because it constitutes a novel form of information content and transmittal. It may also have served as a FET for the MST that occurred during the Cambrian explosion, due to its potential role in the transition to multicellularity (Wang et al., 2021).

PROKARYOTES TO EUKARYOTES

The adaptive radiation and ecosystem transformation that occurred in the Cambrian (and likely also in the Ediacaran) is perhaps the single most physically apparent MST throughout history. The eukaryotes that transformed these ancient ecosystems differed in multiple major characteristics from the prokaryotes that dominated the previous two billion years. At the intracellular level their differences are profound. Unique to eukaryotes are: (1) multiple diploid, linear chromosomes (Goodenough and Heitman, 2014); (2) segregation of chromosomes into a nucleus; (3) mitochondria (Margulis and Fester, 1991); and (4) other structures, such as the endoplasmic reticulum (Butterfield, 2015). 'Eukaryotic' fossil cells are recognizable as larger than contemporary prokaryotes, with evidence of nuclei (Sun et al., 2020). Eukaryotes first appear about 1.6–1.8 BYA, but remain relatively minor components of ecosystems for another billion years, until about 635 MYA when the Cambrian MST occurred (Butterfield, 2000; Knoll et al., 2006; Knoll, 2011).

The Cambrian MST occurred only in conjunction with later biological innovations of complex multicellularity (i.e., plants, animals, and fungi) and meiotic sex (Goodenough and Heitman, 2014), which likely required significantly more Level I and II information than present in early eukaryotes. Hence, the previously described genome expansion in eukaryotes must have played a facilitating role in this MST. Moreover, at least three separate innovations produced mitochondria, nuclei, and diploidy, each of which also played a facilitating role in the Cambrian MST (**Figure 2**). Understanding the ecological impact of eukaryotes may therefore require considering synergies across multiple biological innovations, at least one MET and possibly multiple MCTs acting as FETs, along with biotic and abiotic catalysts.

Mitochondrial Symbiosis

There is strong evidence that mitochondria arose through endosymbiosis between a free-living relative of α-proteobacteria (the endosymbiont) and an early archaeon (Margulis and Fester, 1991), although how this endosymbiosis occurred and whether phagocytosis was involved continues to be hotly debated (Cavalier-Smith, 2009; Lane, 2011; Spang et al., 2015). Nevertheless, the mitochondrial symbiosis is clearly a Fusion MET (Szathmáry, 2015). Interestingly, all existing mitochondria and chloroplast symbioses may trace back to no more than three initiating events (Margulis and Fester, 1991): one for the former, and two for the latter. Lane (2011), a proponent of the "phagocytosis-second" hypothesis, suggests that the improbability of acquiring an endosymbiont without phagocytosis may help explain why eukaryotes arose only once. Another explanation is that the initial stages of such relationships are not particularly advantageous for either participant (i.e., not a MCT), and require additional adaptations to produce significant benefits and persist.

Evolution of the Nucleus

Three competing hypotheses for the origin of the nucleus are: endosymbiosis (Baluška and Lyons, 2018a,b), autokaryogenesis (Lisitsyna and Sheval, 2016), and the "inside out" hypothesis (Baum and Baum, 2014). Under the endosymbiosis hypothesis, possible host cell identities include an ameba (Mereschkowsky, 1905), bacterium (Forterre, 2011), proteo-bacterium (Lake and Rivera, 1994), or archaeon (Margulis et al., 2000), and candidates for the endosymbiont are an archaeon (Lake and Rivera, 1994; Forterre, 2011), spirochete (Margulis et al., 2000), or virus (Bell, 2001; Takemura, 2001). Any of these endosymbiosis scenarios would be a Fusion MET. The other two hypothesized scenarios, however, would not be METs. Under the autokaryogenesis hypothesis, nuclear membranes and closely connected endoplasmic reticulum formed from invaginations of inner membranes of prokaryotes (Lisitsyna and Sheval, 2016). Finally, the inside-out hypothesis posits that a free-living cell ancestral to the nucleus increased its surface area through developing extracellular protrusions (as are common in archaea), which ultimately gave rise to the endoplasmic reticulum and cytoplasm (Baum and Baum, 2014).

One major advantage of nuclei is physically separating the process of translation from the processes of transcription and splicing (Szathmáry, 2015; Lisitsyna and Sheval, 2016). Splicing removes introns from a precursor RNA, leaving only exons, and alternative splicing creates different isoforms from the same gene (Keren et al., 2010). Because splicing is slow compared to translation, the physical separation of these processes appears essential to the completion of splicing before translation (Martin and Koonin, 2006). Eukaryotic gene regulation with introns may be "impossible" without such physical separation (Szathmáry, 2015).

Diploidy

Eukaryotes differ from prokaryotes in being diploid with homologous, but non-identical, chromosomes for at least a portion of their life cycle. Diploidy can arise by endomitosis wherein cells duplicate their genetic material (Tüzel et al., 2001). Cells with non-haploid states quite possibly predate the appearance of eukaryotes, as many extant species of bacteria

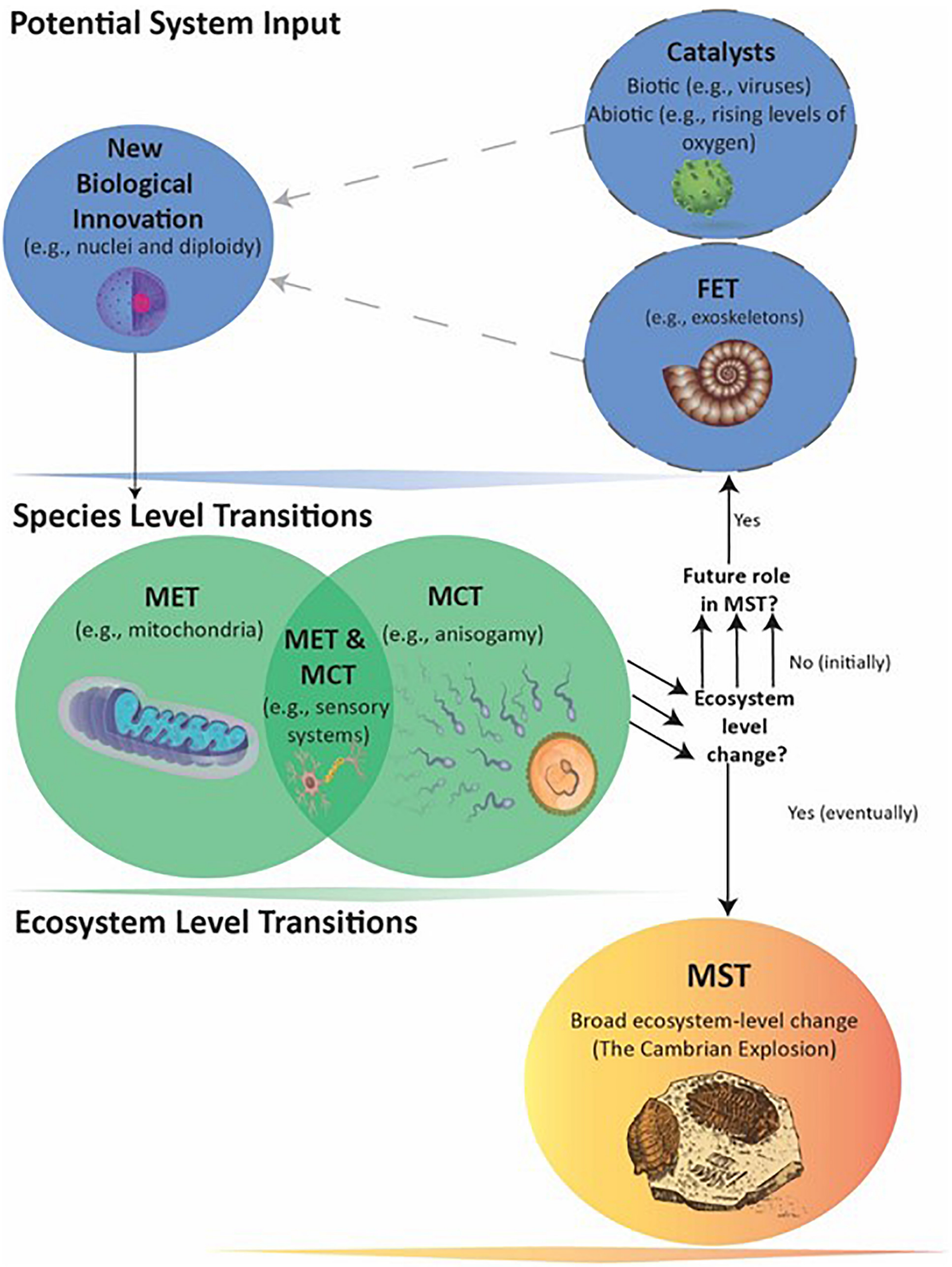

FIGURE 2 | Events leading to the Cambrian MST. The transitions from prokaryote to large eukaryotes with tissues and organs likely involved multiple Fusions and Information Leaps, MCTs, and catalytic agents and events. The multiple arrows indicate that a number of separate Species-level transitions created multiple FETs that eventually made the Ecosystem-level MST possible. The exact order in which the FETs evolved is not conclusively known (see text).

and archaea are polyploid. Indeed, some species routinely have thousands of copies of their chromosome per cell (Soppa, 2014, 2017). Even viruses have been found to package multiple genomes into virions (Chou et al., 2012). Interestingly, although the ploidy of the archaeon *Lokiarchaeota*, which may have been ancestral to eukaryotes, remains unknown, features such as the presence of histones strongly suggests polyploidy (Markov and Kaznacheev, 2016).

Genomes becoming diploid increases storage for Level I information, but doubling a prokaryotic genome alone does not come close to modern-day size differences between prokaryotic and eukaryotic genomes (Bird, 1995). The initial spread of diploidy likely required other competitive advantages beyond slightly more Level I information. Proposed advantages could include mechanisms to repair damaged DNA, and compensating or hiding deleterious mutations on one gene copy with redundant functional copies on the other (Bernstein et al., 1981). In haloarchaea, polyploidy provides increased resistance to desiccation, and is a storage vessel for phosphate in times of scarcity (Ludt and Soppa, 2019). In environments with limited resources and highly competitive haploid organisms, diploidy could be a "last chance" alternative life history strategy where two low-fitness haploids fuse into a competitively enhanced diploid (Jan et al., 2000). Combining diploidy with sexual reproduction can further create greater degrees of variation in offspring due to recombination (Bernstein et al., 1981). Non-haploid states, however, also create costs (Markov and Kaznacheev, 2016) as genome copy number can vary across cells due to random segregation at cell division (Ludt and Soppa, 2019). Accurate segregation, as occurs with mitosis, avoids this cost while retaining the advantages. Indeed, in simulations, mitotic diploidy can be favored over haploidy (Tüzel et al., 2001).

In conclusion, the evolution of eukaryotes with mitochondria, nuclei, and diploidy with multiple linear chromosomes must have required at least three distinct events. However, they all apparently evolved so closely together in time that we may never know which came first and the degree to which one event facilitated a second or third. There are no known intermediate eukaryotes with only one or two of these characters (although some species subsequently lost mitochondria over their evolutionary history; e.g., Karnkowska et al., 2016). One possible explanation for the lack of extant intermediates and the fact that eukaryotes arose only once, despite the continual existence of biodiverse and interactive prokaryotic communities, is that any one of these events alone was not particularly advantageous (i.e., not a MCT), and persisted only when followed by a fortuitous second or third event in quick succession. For instance, a symbiosis between mitochondrion-like α-proteobacteria and host could have required rapid suppression of selfish intragenomic selection in the former in order to persist (Bourke, 2011a). This may have only been possible by "genomically neutering" the mitochondrion through differential movement of essential genes from their genome into linear chromosomes segregated behind nuclear membranes (Nonacs and Tolley, 2014). Ultimately, regardless of whether any of the events that produced eukaryotes qualify as MCTs, the mitochondrial symbiosis nevertheless renders eukaryotes a Fusion MET. Moreover, this MET is a FET for the subsequent Cambrian MST (**Figure 2**).

THE EVOLUTION OF SEX

Sexual reproduction is defined by meiosis followed by syngamy, the fusion of two cells and their nuclei (Santos et al., 2002). Sexual reproduction is found only among eukaryotes and apparently universally so, suggesting it appeared early in their evolution (Cavalier-Smith, 2002). In fact, critical features needed for sexual reproduction possibly preceded the mitochondrial symbiosis. For example, the fusion of gametes is mediated by cell surface glycoproteins, which appear to predate the last common ancestor of eukaryotes and archaebacteria (Cavalier-Smith, 1987). However, these may originate from 'old' viral genes that were horizontally acquired by a previously asexual eukaryote (Valansi et al., 2017). The difficulty in delineating the timelines of specific eukaryote-affiliated events led Szathmáry (2015) to subsume the evolution of sex into a single combined transition from prokaryotes to eukaryotes (**Table 1**). We differ from Szathmáry (2015) in that the origin of eukaryotes is considered not as a single event, but as a possible series of MET(s) and MCT(s) acting as facilitating evolutionary transitions [FET(s)]. Similarly, we consider the evolution of sex a MET and the evolution of anisogamous sexual reproduction a MCT (**Figure 2**), both of which are also FETs. Additionally, viruses appear to have played an important catalytic role through gene transfer.

Our classification of the evolution of sex as a MET follows from three consequences for which sexual reproduction is uniquely responsible: (1) changing the units of selection; (2) producing a novel form of Level I and II information transmission that increases the rates at which beneficial information spreads through populations and deleterious information is eliminated; and (3) altering natural selection by creating or revolutionizing the processes of sexual, kin and intragenomic selection.

Units of Selection

Sexual reproduction is, in a sense, a return to the earliest stages of life on Earth – the era of replicator molecules. Sex with recombination makes genes the units of selection instead of genomes (Jablonka and Lamb, 2006). On a second level of selection, reproduction requires the fusion of two distinct entities (e.g., egg and sperm). With the exception of species capable of self-fertilization, this requires coordination between two individuals and implies that mating pairs are a higher-level unit of selection (Michod, 2011). Clearly, such pairings experience unique selection pressures pertaining to partner choice (Andersson, 1994) and postcopulatory sexual selection (Birkhead and Pizzari, 2002), resulting in novel morphologies and behaviors. The mating pair, however, is not an indivisible individual, such as organelles in eukaryotes. Therefore, one could argue that sex is not a MET because mating pairs do not explicitly generate mating pairs as the propagating units (Calcott and Sterelny, 2011). Nevertheless, the offspring of a mating equally represent both parents. They are also indivisible individuals. Hence, this objection is a quantitative one – an offspring of a mating pair only partially, instead of entirely, replicates the pair.

Information Availability and Management

Sexual reproduction expands the dynamism of heredity. Recombination through sexual reproduction aggregates beneficial mutations and eliminates harmful ones more rapidly than asexual reproduction (i.e., escaping Müller's Ratchet: Bernstein et al., 1981; Fagerström et al., 1998). Although individuals only directly access their own genotypes, their descendants potentially access all the Levels I and II information in populations. Therefore, sexual populations can rapidly create genetic combinations that are adapted to local environments in ways not previously observed in asexual organisms (Lively and Morran, 2014; Sharp and Otto, 2016). For example, recombination allows hosts to develop defenses against more rapidly reproducing asexual pathogens (Lively and Morran, 2014; Sharp and Otto, 2016). This benefit in the Red Queen arms race (Van Valen, 1973) can offset the disadvantage of longer generation times in sexually reproducing eukaryotes relative to asexual prokaryotes. Furthermore, the Level I information contained within an asexual pathogen must compete against the entire Level I information present in the sexual population of its host. Notably, no asexual variant has been observed to out-compete and displace their sexually reproducing conspecific, even in stable environments (Niklas et al., 2014).

Sexual, Kin, and Intragenomic Selection

Sexual reproduction changes evolutionary processes, perhaps more so than any previous innovation. The evolution of anisogamy first creates sexual selection. Fitness now depends on both survival and the ability to secure a mate. Only sexual selection provides a coherent explanation for the many costly and flashy traits that serve primarily to win intrasexual contests or increase attractiveness to the opposite sex, while simultaneously reducing the bearer's survivability (Darwin, 1871; Andersson, 1994).

Sex also revolutionizes kin selection. In completely asexual organisms, individuals functionally become their own reproductively-isolated species. The Levels I and II information in two daughter cells from a fission event are as separated going forward in time as the information in two cells last sharing a common ancestor a billion generations ago. In asexual populations, kin selection may be invoked when genetically similar individuals assort non-randomly (Levin and West, 2017), or social heterosis invoked when heterogeneous groups are the more productive (Nonacs and Kapheim, 2007), although arguably individual-level selection across clonal lineages provides an equally adequate explanation (Nowak et al., 2010). Sex, however, creates more variable and quantifiable classes of kinship and many potential cooperative and competitive interactions predicated on the degree of shared genes, with examples such as nepotism, parent-offspring conflict, siblicide, adaptive suicide and senescence (Forbes, 2005; Bourke, 2011a,b). Interestingly, the evolution of highly eusocial species (i.e., those having morphologically distinct reproductive and worker castes) is attributed to kin selection and has occurred far more often in sexual than asexual species (Bourke, 2011a,b).

Finally, sexual reproduction creates a new level of selection through intragenomic conflict. For instance, a variety of sex-ratio distorting genetic elements exist by biasing the outcomes of meiosis in their favor (Queller, 1997; Burt and Trivers, 2006). When genomes come equally from mothers and fathers, the two halves may not necessarily maximize their fitness in the same way. Parentally imprinting genes (i.e., Level II epigenetics), therefore, can cause differential expression in offspring (Reik and Walter, 2001). In mammals, this results in paternal and maternal genomic conflict within individuals over the amount of resources extracted from mothers during gestation (Haig, 2015).

Major Competitive Transitions and Catalysts

The origin and elaboration of sexual reproduction required a number of critical morphological innovations, including those involved in the fusion of cells and the transition from isogamy to anisogamy. In cellular fusion, sperm and egg cell membranes merge into a single, unbroken one (Clark, 2018). Viral genomes enter host cells by a similar process, made possible by an essential class of proteins (fusexins). Genes coding for these proteins are very similar across viruses and eukaryotes (Valansi et al., 2017). Thus, sexual reproduction appears to be made possible because of horizontal transfer of viral fusogens (Hernández and Podbilewicz, 2017). Without a virus as a catalytic agent, the evolution of sexual reproduction may not have been mechanically possible.

Within multicellular eukaryotes, anisogamous gamete production predominates. It is unknown whether the evolution of complex multicellularity with differentiated tissues needed to precede anisogamy or vice versa (Hanschen et al., 2018). However, it is clear that the first sexually reproducing eukaryotes were likely isogamous (Parker et al., 1972; Yang, 2010; da Silva, 2018). Mathematical models predict that such a state is often evolutionarily unstable. Mutants that tradeoff motility for larger size increase relative fitness through higher offspring survival (Parker et al., 1972), and others with motile gametes can increase their numbers by decreasing individual cell size. The disruptive selection eventually results in large, immobile eggs and motile sperm of minimal size (da Silva, 2018). If there is a further significant transport cost in finding receptive gametes, then two alternative reproductive strategies result: either produce many searching sperm or few eggs waiting to be found (Yang, 2010). Because it is likely that the initial benefits of anisogamy relative to isogamy were great, the evolution of anisogamy is a MCT.

In conclusion, sexually-reproducing eukaryotic species are and have been significant parts of almost every known ecosystem since the Cambrian (Santos et al., 2002; Jablonka and Lamb, 2006; Szathmáry, 2015). It is also notable that among the many metazoan lineages, only bdelloid rotifers have maintained a non-sexual and non-meiotic lifecycle on a geological timescale, through employing alternative methods to generate genetic diversity (Flot et al., 2013). Given the magnitude of sexual species' effects on ecosystems, the evolution of sex is a critical FET composed of a MET and at least one MCT – that together paved the way to a MST.

THE EVOLUTION OF MULTICELLULARITY

Egalitarian and Fraternal Associations

Multicellularity has been defined in a variety of ways, ranging from functionally independent cells clumping as groups to indivisible multicellular entities

(Hammerschmidt and Rose, 2021). Such associations can be "egalitarian" where cells do not derive from a recent common ancestor (Queller, 1997). Cheating, however, can be a serious problem. For example, cooperators could produce costly compounds to stick together as mats and increase reproduction for all cells (Velicer and Yu, 2003). Cheaters not producing the compounds gain these benefits without incurring the costs. They, therefore, increase in frequency until mats collapse to everyone's detriment (Tarnita, 2017). Overall, egalitarian systems may lack stabilizing mechanisms needed to maintain equal intragroup reproduction; severely limiting evolutionary potential (Rainey and Kerr, 2011).

Alternatively, multicellular associations can be "fraternal" where all cells share a recent common ancestor (Queller, 1997). In clonal associations, direct and indirect fitness are indistinguishable by genetic relatedness (Bourke, 2011a; West et al., 2015). Therefore, the evolutionary process favoring cooperation in clones may be conceptualized as either kin, group or lineage selection. Consider a multicellular clone with reproductive division of labor across germline and somatic cells that is competing with another where all cells reproduce. If the former produces more total offspring, that clone should increase in frequency.

Interestingly, mutations that diversify growing fraternal clones can create a kin-selective scenario that evolutionarily stabilizes reproductive specialization. If cells divide at unequal rates, the most rapidly dividing lineages will accumulate more divergent mutations. At this point, associations are no longer completely identical clones and distinctions arise between direct and indirect fitness. Ideally, each genetically unique cell or lineage should strive to reproduce while suppressing all others. If none can dominate all competitors, then the stable solution favors a consensus 'second best' relative as the reproductive (Reeve and Jeanne, 2003). The competing lineages will likely all have the same closest relative – those cells that divided the least and accumulated the fewest mutations (Queller, 2000). This group of cells would, therefore, be acceded "virtual dominance" over reproduction without needing an intrinsic ability to suppress competitors (Reeve and Jeanne, 2003). Although looking like a 'cheater,' the virtual dominant would be tolerated and favored to become the germline in the maturing clone (Veit, 2019). Indeed, in many animals, some cells are segregated very early in development and these become the gamete producers (Kumano, 2015).

Simple Multicellularity

The transition from a population of cells into an integrated multicellular organism requires several developmental innovations, such as cells adhering to one another and exchanging signals to coordinate activities (Niklas, 2014). Mathematical models suggest that a wide variety of factors favors such transitions from a solitary cell to a grouped state (Staps et al., 2021). This would be Stage 1 in the evolution of multicellularity, where each cell directly benefits from being in a larger association (Hammerschmidt and Rose, 2021). The evolutionary transition to Stage 2 occurs when group-level reproductive success becomes dependent on cooperation between cells, and entire assemblages act as multicellular individuals (Hammerschmidt and Rose, 2021).

Nevertheless, fossil records provide little evidence that early multicellular organisms had significant competitive advantages (MCTs) over single-celled ones or greatly altered ecosystems (producing a MST). Indeed, many extant species with Stages 1 and 2 multicellularity still retain significant single-celled portions of their life history, or readily switch back and forth as environmental conditions change (Staps et al., 2021). Moreover, the simplest possible multicellular associations – rudimentary bacterial mats – changed little over their initial billions of years of life. Thus, the first appearance of multicellularity was neither a MCT nor a MST. Simple multicellularity is also not considered a MET (**Table 1**) because it is neither a fully formed, higher-level individual nor a novel form of information storage or transmission. Simple multicellularity would appear in **Figure 1** only as a 'new biological innovation' that was needed for the eventual MET of complex multicellularity (Stage 3: with obligate ontogeny of differentiated tissues; Hammerschmidt and Rose, 2021).

Abiotic Catalyst

Complex multicellularity has arisen independently in multiple eukaryotic lineages, with a common feature: organisms' overall energy demands are met by subsets of cells dedicated to meeting them. A critical abiotic catalyst for this transition (**Figure 1**) is a sufficient level of free oxygen to support high-energy metabolisms based on oxidative phosphorylation. Earth's history reflects two periods of rapid oxygen increase: (1) the Great Oxygenation Event (∼2.5–∼2.0 Gya), when oxygen first became consistently available in low but biologically significant concentrations; and (2) the Neoproterozoic Oxygenation Event (∼0.8–∼0.54 Gya) when concentrations rose to near present levels (Och and Shields-Zhou, 2012; Lyons et al., 2014). Significantly, complex multicellular forms only appear in the late Neoproterozoic (Nursall, 1959; Budd and Jensen, 2000; Lyons et al., 2014). Thus, this second event was probably an important abiotic catalyst for the evolution of complex multicellularity (**Figure 1**).

Complex Multicellularity

In complex multicellular species with differentiated tissues and organs, development follows the fraternal route. Life history proceeds through a single cell stage, most commonly the fusion of two gametes, with clonal growth into the mature state (Bourke, 2011a). That complex multicellularity is closely tied to sexual reproduction may be because such organisms necessarily reproduce much more slowly than unicellular organisms, and relying on purifying selection to remove deleterious mutants from asexual lineages is a huge disadvantage. Sexual reproduction, therefore, allows a more rapid purging of deleterious mutations through recombination and greater genetic variation in offspring for adapting to changing environments or co-evolutionary arms races with pathogens (Fagerström et al., 1998).

Waddington's (1957) epigenetic landscape seemingly answers the question of how reproductive specialization is maintained across cell lineages. Somatic differentiation with Level II epigenetic modification limits lineages to terminal fates (Pacheco et al., 2014). Mechanistically, this prevents competition arising with virtual dominant germlines. Thus, non-germline lineages are freed to, for example, evolve as sensory and neural systems that receive and transmit information, or become specialized in provisioning and transporting of metabolites, nutrients and gasses to maintain overall homeostasis beyond what is possible by diffusion (Knoll and Lahr, 2016).

A strict reproductive segregation, however, is far from the case across all multicellular species. Many cnidarians, for instance, display remarkable abilities to transdifferentiate, seemingly including converting somatic cells into germ cells (Gold and Jacobs, 2013). Medusozoan cnidarians are even capable of "degrowing" gonadal structures or reverting to asexuality (Hamner and Jenssen, 1974; Piraino et al., 2004). Similarly, plants do not segregate germlines from somatic lineages, but rather derive germ cells from somatic lineages (Kawashima and Berger, 2014). Therefore, a Waddingtonian view of canalized germ cells cannot be absolutely necessary for the evolution of tissues and organs. Furthermore, cellular 'cheaters' (e.g., cancers) are much more common in bilaterian clades (Aktipis et al., 2015), correlating with evolutionary losses in cell totipotency as their tissues differentiate. It is paradoxical that cancer is rare or absent in cnidarians and ctenophores where somatic cells can quickly revert to being gametic and thus would seem to be especially vulnerable to mutations for cheating. Analogs to present-day animal oncogenes appear to have evolved well before the appearance of bilaterians (Trigos et al., 2019), but their causative propensities are most evident in taxonomic clades where cell sterility is normally obligate and not facultative.

Ultimately, although all stages of multicellularity create higher-order associations, only Stage 3, tissue-level cellular differentiation truly constitutes a MET (**Table 1**). It is when the component parts of an organism must developmentally specialize that there is a fully formed, higher-order "individual."

An Evolutionary Scenario

Rising oxygen concentrations in the Neoproterozoic increased viability of aggregations of mitotically dividing eukaryotic cells, and a potential scenario emerges for evolution of complex multicellularity. Aggregations create opportunities for innovative cooperation and division of labor across cells (Wolpert and Szathmáry, 2002). Freeing cells from having to provide their own energetic and metabolic needs allowed diversification and specialization. The actual phenotypic innovations vary across groups. In plants, MCTs like vascular tissue enabled colonization of the terrestrial environment and the evolution of larger sizes (Stebbins, 1969) and flowers provided the opportunity to co-opt animals into being vectors for fertilization. In animals, the evolution of nervous systems repurposed cells for storage and transmission of information (Jablonka and Lamb, 2006). Level III Learning allowed the evolution of behaviors requiring predictive ability and flexibility such as parental care, territoriality, mate choice and sociality. New evolutionary rules for behavioral interactions such as direct and indirect reciprocity (Maynard Smith, 1982; Nowak, 2006) became important processes within natural selection. Given that multiple separate lineages evolved complex multicellularity almost simultaneously while using different genetic and developmental mechanisms, it seems these were METs merely waiting for the right set of opportunities to arrive.

By conservative estimate, complex multicellularity with tissues and organs emerged at least six times in sexually reproducing eukaryotes: animals, plants, red algae, brown algae, and at least twice among fungi (Bernstein et al., 1981). Observing the range of shared commonalities across lineages would lead to better understanding of how such a variety in the same category of MET occurred. Unfortunately, inferences are limited because the evolution of development is well studied only in lineages of bilaterian animals and higher plants.

The appearance of complex multicellularity fundamentally changed the planet; altering environments and creating entirely new ecological niches such as vertical communities of kelp forests (Teagle et al., 2017). Swimming animals in the oceans increase biomass export to the deep sea, sustaining the oxygenated state of the oceans (Butterfield, 2018). Land plants potentially stabilized climatic conditions throughout the Phanerozoic via their root systems increasing weathering of rock (Ibarra et al., 2019). Plant leaves alter the water cycle via transpiration and are responsible for the majority of global evaporation (Schlesinger and Jasechko, 2014; Wei et al., 2017). Where bacterial mat stromatolites once dominated, the evolution of animals restricted their range to stressful environments free from grazers (Walter and Heys, 1985; Sheehan and Harris, 2004). The many significant and continuing ecosystem-level impacts of complex multicellular species certainly qualifies as a MST.

EVOLUTION OF EUSOCIALITY

The term "eusociality" is applied to group-living species that divide reproductive labor, cooperatively care for offspring, and have overlapping generations of parents and adult offspring (Batra, 1966; Wilson, 1971). This broad definition covers a continuum of species, without a clear point of demarcation as to where any major transition happened (Bourke, 2019). Recently, the MET is proposed to be crossed when a 'queen' caste monopolizes reproduction, with an obligately sterile, morphologically-distinct worker caste (Boomsma and Gawne, 2018).

Contrary to current opinion (Maynard Smith and Szathmáry, 1995; Queller, 2000; Szathmáry, 2015; West et al., 2015; Boomsma and Gawne, 2018; Bourke, 2019), we propose that eusociality is not a MET (**Figure 3**). We argue that eusocial species neither fundamentally alter how information is stored and transmitted across individuals, produce a new level of individual, nor create new or enhance existing mechanisms within natural selection. However, many eusocial species are remarkably abundant and ecologically dominant, qualifying them as a MCT. In some cases, the evolution of more elaborate eusocial societies (e.g., ambrosia beetles and fungus-garden ants) may have required the catalyst of competition and mutualism with viruses, bacteria and fungi (Biedermann and Rohlfs, 2017) (**Figure 3**).

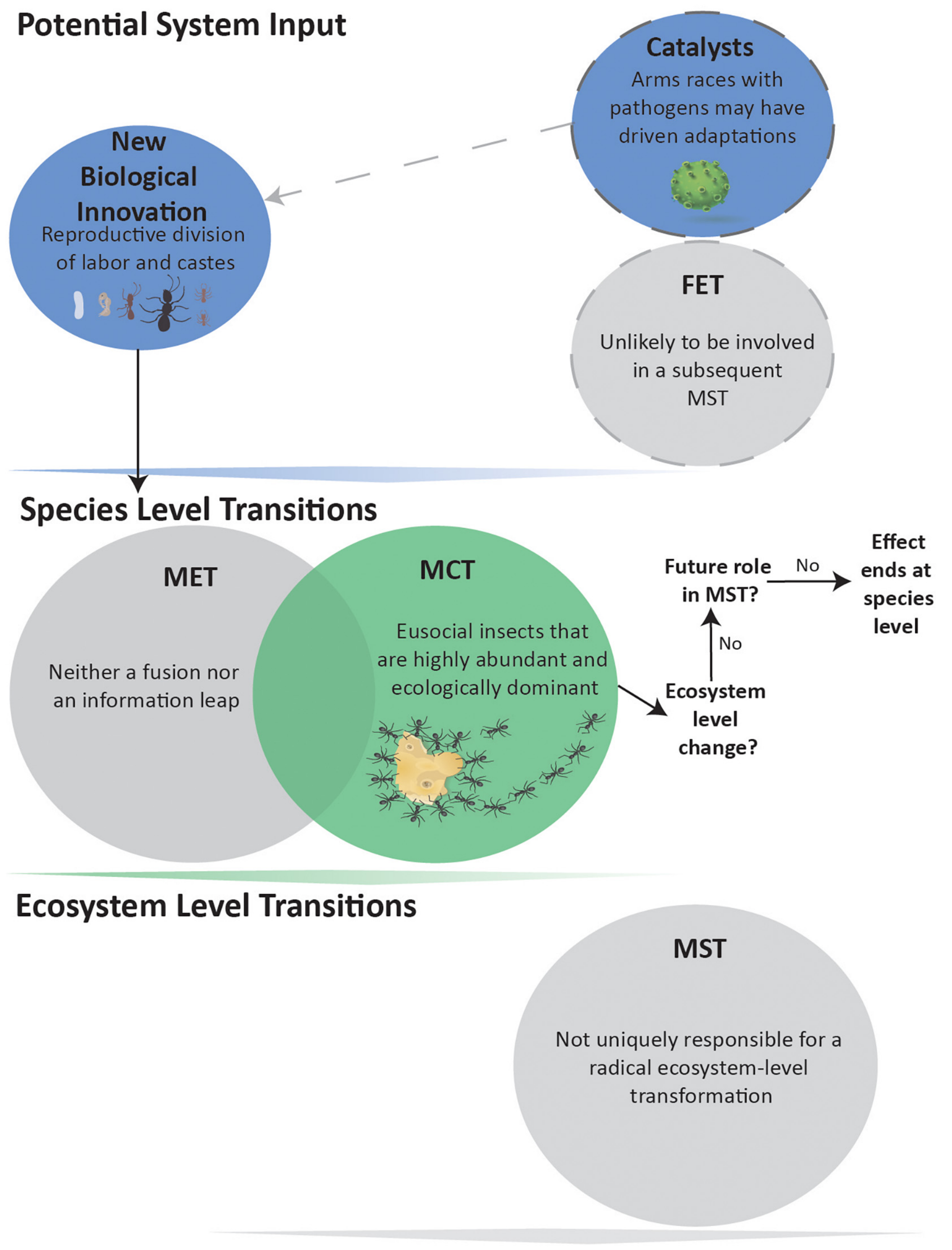

FIGURE 3 | Eusocial evolution not leading to a MST. Evolution of morphologically distinct castes and reproductive division of labor is considered a MCT due to the abundance of social insects. These biological innovations are neither Fusions nor Information Leaps and, therefore, not a MET. The eusocial insect MCT has arguably not produced an Ecosystem-level MST, and shows no indication for being a FET in any such impending event.

Level I and II information in eusocial species is stored and transferred across generations no differently than in other sexual species. No matter how genetically diverse a group might be, any given offspring reflects a genetic bottleneck of no more than two parents. Eusocial groups make substantial use of Level III and IV (Iconic) information as multiple individuals rely upon chemical trails to track resources, activities of neighbors, or looming threats to a far greater degree than any single individual. However, chemical communication did not originate in eusocial species, and thus is not a qualitative Information Leap. Moreover, Level III and IV information is ephemeral across generations. The same structures and trails may be used across generations within a colony, but its dispersing offspring carry along none of this information. It is likely that parental birds pass more Level III information to their chicks than any social insect colony passes to a descendant colony. Overall, therefore, all known eusocial species fail the MET criterion of adding or expanding levels of information transmittal or storage.

Eusocial species in which individuals are so morphologically differentiated that group living is obligate would seem to strongly suggest a transition to a higher level of individuality (Boomsma and Gawne, 2018; Bourke, 2019). An alternative view, however, is that eusociality is a modification of complex multicellularity where non-reproductive worker castes are analogous to somatic tissue specializing in maintaining overall homeostasis. This diffuse 'worker caste organ' differs from others like hearts and kidneys in having regions of considerably weaker cell to cell adhesion and higher within-tissue genetic heterogeneity.

Evolving a genetically heterogeneous worker caste organ certainly qualifies as a biological innovation. It is a novel way to create an "extended genotype" that specializes in nurturing the gonadal tissue. Thus, rather than care for and raise their offspring directly, eusocial parents cooperate to create tissue (i.e., workers) that provides food, care and protection to the next generation of reproductive offspring. That worker castes evolved as purposeful constructions through parental manipulation is empirically supported across facultatively eusocial species (Kapheim et al., 2012, 2015; Rehan et al., 2014).

Some (e.g., Boomsma and Gawne, 2018) have included "irreversibility" as a criterion for METs; that is, higher levels of individuality cannot dissolve back into their antecedent parts (Bourke, 2011a, 2019). Although we do not invoke irreversibility in our MET definition, it is nevertheless worth briefly discussing whether eusociality meets this criterion. This is certainly not the case in eusocial species without morphologically distinct castes. Even in genera where group living is common, species can revert to solitary life histories as seen in primitively eusocial bees (Danforth et al., 2003; Cardinal and Danforth, 2011) and paper wasps (Liebert et al., 2005). In contrast, no reversals to solitary living are documented in species with obligate, morphologically differentiated castes (Boomsma and Gawne, 2018). However, species can evolutionarily lose entire morphological castes. Many inquiline parasites of other social species lose their worker castes (Sumner et al., 2003). Conversely, numerous species have lost the queen caste and workers have regained full reproductive capacities (Rabeling and Kronauer, 2013). Although such species continue to live in groups - as solitary living may simply be a poor strategy in a very competitive world - there seems no intrinsic reason why single individuals could not survive and reproduce on their own, under the right conditions. Eusocial species seem to fail, at least hypothetically, as a new level of irreversible individuality.

In terms of processes within natural selection, eusocial species can be affected by direct, kin and group selection (Trivers and Hare, 1976; Nonacs, 1986, 2017; Wilson and Hölldobler, 2005; Bourke, 2011a; West et al., 2015). However, the evolution of eusociality neither originated these processes nor qualitatively altered their operation relative to solitary species. Therefore, eusociality also fails this final possible MET criterion.

We therefore conclude that the evolution of eusociality better fits the category of MCT – a novel innovation that confers significant selective advantages. For example, eusocial species are dominant community members by biomass across a variety of non-arctic habitats (Wilson, 1990, 1992; King et al., 2013). Of the approximately 900,000 known insect species, ants and termites account for about 2%, yet constitute over 50% of insect biomass (Wilson and Hölldobler, 2005). This raises the question of whether species or clade-level abundance is enough to be considered a MST.

The evidence is not conclusive on the effects of eusocial species at ecosystem levels. The first appearance of eusocial termites, bees, wasps or ants has not obviously correlated with pronounced turnovers in fauna or flora. For example, ants first appear in the fossil record about 100 MYA (early Cretaceous), with molecular data suggesting a mid-Jurassic origin (140–168 MYA), but ants become ecologically significant only much later in the Eocene (Moreau et al., 2006). Furthermore, if eusocial species are not present, it seems that their niches within ecosystems are filled by other organisms. Pristine Hawaiian ecosystems, with no native eusocial species (Wilson and Holway, 2010), do not appear fundamentally different in their construction from mainland ones. In summary, eusocial species fall into a gray zone of whether their abundance and ecological persistence is enough to qualify them as a MST. From our perspective, it is not (**Figure 3**). Moreover, current eusocial societies do not appear to have any biological innovations whose downstream consequences are likely to act as a FET for a future MST.

Finally, regardless of whether eusociality is considered a new 'individual' (MET and MCT) or a new type of 'organ' (MCT only), the analogy of workers to somatic cells reveals an interesting parallel to cancers. Cancer or cancer-like cells are observed across many taxonomic groups, but far more commonly in sexual species that segregate gamete production into small populations of germline cells (Aktipis et al., 2015). Thus, cancer can be viewed as a within-individual cheater that rejects the imposition of lost cellular totipotency. In the eusocial hymenoptera, queens are the segregated germlines. Parasitic species reproducing at host expense have evolved in all the social hymenopteran groups (Wilson, 1971; Schmid-Hempel, 1998). Their evolution follows Emery's rule, where social parasites likely originally evolve as a within-species alternative reproductive strategy that rejects imposed sterility (Wilson, 1971) – the equivalents of terminal "social cancers" (Oldroyd, 2002). In contrast, termites are far more indefinite in their developmental trajectories and can 'dedifferentiate' from becoming a worker and instead become a reproductive (Wilson, 1971). Unlike social hymenoptera, termites have no known cancer-like social parasites (Schmid-Hempel, 1998). Termites it seems, are like cancer-free cnidarians while hymenoptera are like cancer-prone vertebrates.

HUMANS

Human Spoken Language

Human language expands the communication of information and intentions between individuals, and is considered a

MET (**Table 1**). Language, however, is found across many other species. Baboons and great apes can transmit and understand sophisticated and subtle concepts (Seyfarth et al., 2005). For instance, apes can communicate through gestures and vocalizations that functionally reference the environment, objects or behaviors (Byrne et al., 2017). Further, individuals actively alter gestures and vocalizations depending on social contexts and targeted audiences (Schel et al., 2013; Byrne et al., 2017). For example, chimpanzees make alarm calls consistent with criteria for intentional signaling when exposed to realistic model snakes, and they target warnings toward naïve individuals unaware of the threats (Schel et al., 2013). Hence, human spoken languages are increasingly viewed as quantitatively rather than qualitatively different from languages of other species. Circumstantial evidence suggests that archaic hominids exhibited communication that was conceptually very similar to that of modern humans (Harris and Bullock, 2002). Given that present-day humans are innately able to learn any language, fairly advanced communication probably predated the dispersion of modern *Homo sapiens.*

Human languages enable social groups to collate greater amounts of Level III information across generations than possible in any individual's lifetime. Transmission of information occurs vertically (from parents to offspring), horizontally (peer to peer) and obliquely (non-parental exchanges across generations), and can occur between individuals that never met or even lived at the same time (Cavalli-Sforza and Feldman, 1981; Jablonka and Lamb, 2006; Danchin and Wagner, 2010; Navarro et al., 2018). This makes cultural evolution a powerful and swift process that heightens humans' ability to adapt, modify and construct their environments relative to non-human species (Feldman and Laland, 1996; Jablonka et al., 1998; Mesoudi, 2017). Furthermore, adaptive cultural innovations are not dependent on occasional random beneficial mutations. Instead, innovations can intentionally arise through trial-and-error learning or as unique combinations of several existing traits (Creanza et al., 2017).

Specific regions in brains are associated with cognition and language ability (Kaup et al., 2011), and size positively correlates with functionality across species (Kotrschal et al., 2013). Large brains relative to body size impose massive metabolic costs at the expense of the body (Kuzawa et al., 2014). Offsetting such a cost in hominids are the benefits of information-rich language that larger brains enable, likely allowing social groups to solve a variety of ecological challenges (González-Forero and Gardner, 2018). Increased cognitive abilities also meant that groups could more effectively cooperate (Herrmann et al., 2007) and practice indirect reciprocity (Nowak, 2006). Indeed, there is no evidence for human language devolving in any ancestral populations, indicating strong positive selection for maintenance (Maynard Smith and Szathmáry, 1995; Szathmáry, 2010).

Therefore, humans' large brains with enhanced language centers are a MCT, and enabled the MET of advanced languages that greatly expanded the amount and availability of Level III information. However, dramatic changes in ecosystems and large-scale environment modifications that qualify as a MST still awaited another innovation (**Figure 4**).

Symbolic Language

Both a MET and MCT, human language is also a critical FET enabling the emergence of Level IV (Instructional) information as inscribed symbolic representations (**Table 2** and **Figure 4**). Information stored in abiotic formats adds another highly efficient method for passing non-genetically inherited cultural traits vertically, horizontally, and increasingly obliquely (e.g., book author – living or dead – to reader) (Feldman and Cavalli-Sforza, 1976; Jablonka and Lamb, 2006; Danchin and Wagner, 2010). This potentially immortalizes, in amount and accuracy, the information that can be transferred from past to current and future generations. Humans are the only known species to store and access extraordinary amounts of Level IV (Instructional) information.

The capacity for symbolic representation of language is critical for the emergence of technological innovations that expanded the realized niche for humans exponentially and paved the path to a global MST. We proliferated across every continent and environment on Earth while substantially impacting these ecosystems. One example of inscribed language producing global-altering information and technology is the very existence of the discipline of evolutionary science and the systematic study of life itself. Humans are uniquely able to understand how evolution works. This creates the novel opportunity to fundamentally alter or even void the processes of natural selection (Stock, 2008; Ghiorghita, 2020). Plants are intentionally manipulated to be more nourishing and harvestable, and animals domesticated for food and service. Through technological advances, humans alter or eliminate important natural selection factors that historically affected our species, such as predators and disease. Sexual and kin selection likewise may be diminishing in importance. For example, sperm donation and surrogacy allows individuals to pass on genes independent of having a mate or being able to become pregnant. Selectively editing human genomes is not inconceivable (Cai et al., 2016). Fetal genetic testing and potential genome editing may directly change population-level allele frequencies, with associated serious ethical concerns.

Human-produced technology creates a virtually limitless capacity for storage and retrieval of cultural information. Online cloud storage placing information in centralized and easily accessible repositories greatly reduces the need for physical storage space. It is estimated that the four major cloud-based storage systems: Google, Facebook, Amazon and Microsoft, have 1.2 million terabytes of information stored (Dastbaz, 2019). Cloud storage also drastically shifts information access, making a great multitude of data types simultaneously available from anywhere on the planet. This can increase the rate of cultural evolution and act as a homogenizing factor across cultures. Increasingly in the future, technologies, cultural institutions and biotic life will be locked into a continual process of co-evolution, where the selective processes involved will differ from those that heretofore affected the evolution of all life on earth. Overall, these changes will likely also influence biological

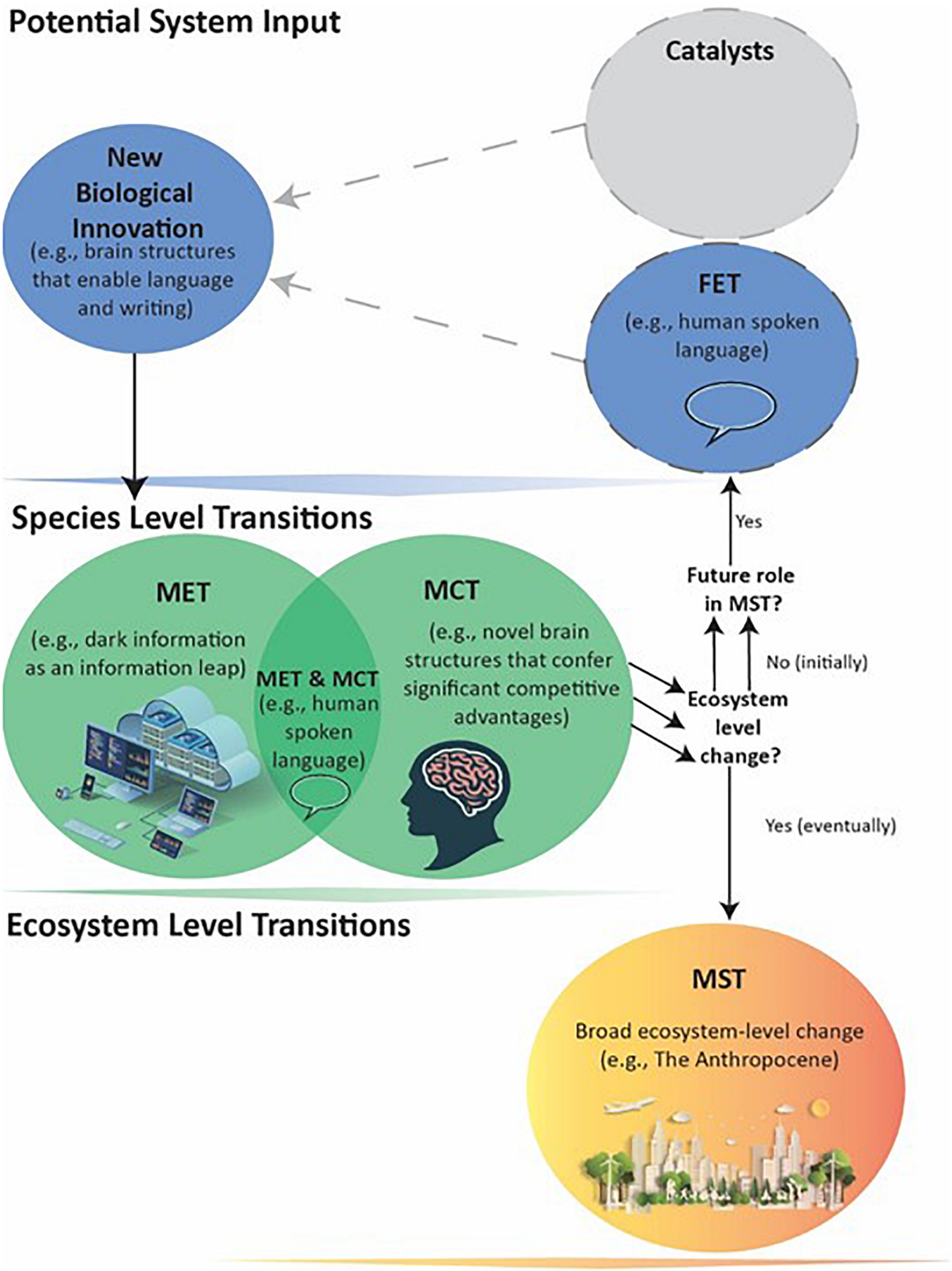

FIGURE 4 | Human evolution leading to a MST. Humans produced broad Ecosystem-level effects through a combination of METs (e.g., spoken language in a Level III Information Leap) and MCTs (e.g., increased intelligence with larger brains). These became separate and essential FETs, as indicated by the multiple arrows, that eventually enabled the Level IV Information Leap MET of storable symbolic language and the MST. As MSTs can 'set the stage' for future METs, MCTs, and MSTs, it is thus possible that human-created technologies (e.g., robotics combined with Level V artificial intelligence) will lead to a future major transition that melds the biotic and abiotic into a new individual.

evolution across the planet for millions of years (Gowdy and Krall, 2014). Therefore, humans' explosive population proliferation and associated profound ecosystem effects bear the hallmarks of a MST.

Level V: Dark Information

Out of the technological advancements made possible through inscribed language a new level of information has emerged. Level V is information generated and transmitted by abiotic entities, such as computers. The process from input to generated output cannot be replicated except by computer, and therefore may remain inscrutably dark. Examples of such dark processes are increasing rapidly. Modern deep learning algorithms form representations of multiple layers of features of data without specific instructions regarding what to learn (LeCun et al., 2015). Human-directed computation and unsupervised machine learning explore difficult questions by searching the World Wide Web in medicine, mathematics, physics, economics, and engineering (Obermeyer and Emanuel, 2016; Sanchez-Lengeling and Aspuru-Guzik, 2018; Brummitt et al., 2020). Collection and analyses of internet searches and activity, phone, text and email communications, and GPS locations inform product marketing, terrorism prevention, and global markets (Greenwald and MacAskill, 2013; Einav and Levin, 2014; Xu et al., 2016). Artificial intelligence designs autonomous machine learning algorithms to diagnose cancer (Song et al., 2017) or control self-driving cars (Hecker et al., 2018). Further, algorithms are increasingly implemented to strategically decide and place user-specific advertisements, as well as generate novel content which can potentially perpetuate cultural features such as fashion trends (Lee and Cho, 2020). Finally, machine learning programs are expanding to the point of generating novel problem-solving algorithms without any added human input (Real et al., 2020). Thus, a key defining feature of Level V Dark information is the ability to create new information without any direct involvement of a biological entity.

In summary, human language and the brain that produces it qualify as a MET and MCT, respectively. The innovation of advanced forms of language increased and changed information content and transmittal, providing humans a critical competitive advantage (Jablonka and Szathmáry, 1995). Spoken language also served as a FET for the emergence of Level IV instructional information, the requisite component for our current ecological transformation and production of a MST (**Figure 4**). The ability to accumulate and immortalize Level IV information through stable storage systems and transfer it across generations enables development of increasingly advanced technologies and the advancement of knowledge, such as our fundamental understanding of evolutionary processes. The technological innovations made possible our manipulations of environments across every part of the globe, perhaps with irreversible ecosystem-level changes. Our understanding of natural selection presents the unique possibility of both exempting ourselves from its dictates and directing evolutionary changes in other species. Ongoing technological innovations also created for the first time: Level V Dark information, which in the span of a few decades is strongly affecting human interactions and societal institutions. It is too early to predict where the expanding Dark Information will lead, except that it will likely be quite impactful. One increasingly realistic possibility is that human-facilitated artificial intelligence, robotics, and Level V information will themselves be FETs for a future symbiotic MET that creates a new level of individual as a combination of biotic lifeforms with abiotic technologies.

DISCUSSION

Today's biotic world differs greatly from that of four billion years ago. In this timespan multiple Major System Transitions (MSTs) created or significantly altered ecosystems, ranging from the origins of life to humans' large-scale modification of nearly every extant biome. Within this history, Maynard Smith and Szathmáry (1995) defined Major Evolutionary Transitions (METs) as leaps in organismal complexity forming higher-level 'individuals' or creating novel forms of information storage or transfer, regardless of any resulting ecosystem impacts (Szathmáry, 2015). Analogously, we classify as Major Competitive Transitions (MCTs) biological innovations that produced significant direct-fitness advantages within lineages, such as shelled eggs or endothermy (Huxley, 1942; McShea and Simpson, 2011), regardless of their broader ecosystem impacts.

Not all METs and MCTs result in a MST (**Figure 1**). We can distinguish those that result in or facilitate MSTs through thought experiments. Would today's world look substantially different if a proposed MET or MCT failed to happen? For instance, the first two METs – culminating in a functioning prokaryotic cell with DNA (Szathmáry, 2015; **Table 1**) – are clearly essential precursors to every current ecosystem. If Level I information had remained RNA-based, genome size and total information content would be extremely limited. Without viable and replicating cells, no greater organismal complexity would be possible. Thus, the evolutionary events that led to cells with DNA genomes either produced MSTs or were critical for facilitating MSTs.

It is equally clear that if multicellular, sexually-reproducing eukaryotes with tissues and organs did not evolve or vanished, the remaining ecosystems would be transformatively different. History further suggests their current niches would not be easily refilled. For example, prokaryotes have not shown evidence of evolving multicellular complex beings. Similarly, early eukaryotes required perhaps a billion years, and multiple facilitating events, before the Cambrian MST occurred (Butterfield, 2000; Knoll et al., 2006; Knoll, 2011).

The long delay in transforming a world composed of algal mats to one full of plants and animals illustrates the conceptual importance of "facilitating evolutionary transitions" (FETs). These are innovations that are necessary, but insufficient alone, to initiate a cascade of events that culminates in a MST (**Figure 1**). For instance, the evolution of various sensory systems that conferred significant direct-fitness advantages (MCTs) may have facilitated a massive coevolutionary burst between predators and prey (McMenamin, 1988; Parker, 2011), which was a causal factor for the Cambrian MST. Another FET for the Cambrian MST was the evolution of sexual reproduction, which unleashed sexual selection and revolutionized kin selection. Finally, all these events happened only in eukaryotic lineages, suggesting this more complex cell type was critical for a MST (**Figure 2**). It is likely that without these FETs, there would not have been a MST.

Another proposed MET in the timeline of the history of life is the evolution of eusocial species (**Table 1**), but we argue that eusociality is neither a Fusion into a new level of individual nor an Information Leap. The spectacular abundance of eusocial species across ecosystems has the hallmarks of a MCT (Wilson, 1990), and it may or may not be a MST (**Figure 3**). Certainly, if all eusocial insects disappeared there would be immediate ecosystem-level effects. Indeed, some species inhabiting unique niches might never be replaced; such as leaf-cutting, fungus garden ants. On a longer time-scale, however, the effects may be transitory, which would suggest that eusocial species are not uniquely responsible for the state of the ecosystem. Consider that termites are extremely important members of decomposing communities. If termites had never evolved, however, it would seem extraordinarily likely that decomposing MCTs would have evolved in other non-eusocial lineages. The world would not today be covered 100 meters deep in dead wood. In an analogous real-world experiment, 67 million years ago all large terrestrial vertebrates were likely dinosaurs. From that ecosystem, one might conclude that dinosaurs were a MST. Yet the dinosaurs turned out to be ecologically replaceable by mammals, when they were given the chance. Thus, being abundant and ecologically dominant over a long time might qualify as a MCT (for dinosaurs, birds and mammals the MCT innovation could be endothermy), but would not necessarily imply that this group was responsible for an ecosystem transformation (a MST).

We argue that such a thought experiment validates humans as a MST (**Figure 4**). Human behavior and activity over the last 10,000 years has substantially altered the world's ecosystems and even changed the planet's climate. Humans have also created a new ecosystem we can call the 'technology biome.' This novel ecosystem makes available to its most important inhabitant food, water and shelter, and buffers them from the majority of environmental extremes, predation and disease. It alters the selective processes that do or do not operate. Historically, there is no evidence of anything remotely similar. Therefore, should we disappear like the dinosaurs, it is unlikely that an extant species is currently waiting in the wings to replace us, and our self-constructed niche would soon permanently evaporate.

The key element in driving a human-centered MST is almost certainly the tremendous expansion of Level IV Inscribed instructional information. This continues a pattern where significant expansions in the amount of information available to organisms at any level may be critical drivers of MSTs.

One such driving event was switching Level I Encoded and Level II Epigenetic information from RNA to DNA. DNA can be 'Functional,' where its effects are under natural selection (Graur et al., 2015) or 'Rubbish,' where its effects are not under selection, but there can be individual-level costs of copying it or costs of meiotic driving elements (Burt and Trivers, 2006). The eukaryotic MET created, probably as a byproduct, genomes that appear particularly susceptible to collecting Rubbish (Graur et al., 2015). Diploidy and sexual reproduction create opportunities for intragenomic conflict that favors proliferation of transposable elements (Werren, 2011), and nuclei act as accumulators of horizontally transferred DNA (Nonacs and Tolley, 2014). Over the long-term, there can be evolutionary advantages to genomes enlarged by Rubbish, such as when mutations convert it into beneficial Functional DNA (Werren, 2011). Sexual reproduction in eukaryotes further increases the transmission of beneficial acquired genes throughout populations and more rapid elimination of harmful mutations (Lively and Morran, 2014; Sharp and Otto, 2016). Enlarged genomes can house additional Functional DNA brought in by transposable elements and viruses (Werren, 2011). It seems likely, therefore, that without all the processes that provided the raw material for creating novel Functional DNA, the evolution of higher plants, animals and fungi may have been impossible. Thus, growth in genomic information must have also facilitated the Cambrian MST.

Nevertheless, every new gene is a piece of information and theory predicts that more information is only valuable if its benefit exceeds the costs in gaining it (Stephens, 1989). This logic should also apply to gene number. Every additional functional gene adds costs for replication, making the product it codes for, and the possibility of harmful mutations. Diminishing returns with constant investment costs would set an upper bound on how much Level I information a genome could contain. Although this limits potential morphological complexity, increased levels of behavioral complexity can harness information not residing in the genome – i.e., Levels III–V. For example, humans are a MET, have undergone MCT(s), and have produced a MST – all achieved with fewer functional genes than a water flea has (Colbourne et al., 2011).

It is our extraordinarily large brain that is the MCT which gives humans access to enormous amounts of Level III and IV information and has manufactured the machines that make Level V information. A consequence of this information is that the majority of modern-day humans have exited natural ecosystems and live to varying degrees in our created technology biome. Is another MET possible in this new ecosystem? Although highly cooperative, humans are still far too individualistic to fuse into a higher level of individual, but our increasing reliance on technology could be early in the transition process for an obligate interspecific mutualism MET (**Table 1**). Currently, most humans rely on technology for survival and reproduction and likewise this technology would not exist or replicate without humans. In this case, the 'mutualism' is between a biological entity and an increasingly sophisticated abiotic entity. However, whether a machine can become part of an interspecific mutualism depends on the definition of 'alive.' It is conceivable that combining robotics with AI could produce machines capable of learning, gathering resources, and replication. Such entities would certainly be as sentient as bacteria and equally or more capable of evolving. The more salient question might be whether self-sustaining and self-replicating abiotic life forms would gain any benefit from mutualisms with biological ones.

By considering all the potentially interacting elements involved in producing a MST (**Figure 1**), it is clear that events can simultaneously fall into more than one category (MCT, MET, and FET). Similarly, categories can have variable numbers of events in them. Thus, the number of members in a category could imply something about the evolutionary likelihood of a given event. If obligate interspecific mutualisms are a type of MET (**Table 1**), then thousands of such METs have occurred throughout history. This strongly suggests that interspecific mutualism is not a particularly difficult problem to solve. Similarly, multicellularity and eusociality have both evolved independently multiple times, although quite a bit less often than interspecific mutualism, which seems at odds with a hypothesis that kin selection and high relatedness is a driving similarity across METs (Bourke, 2011a; West et al., 2015).

Of further interest is that not all the METs in the same category have had similar degrees of ecosystem-level impacts. For example, plants, animals and fungi are far more ecologically impactful than red algae although all are multicellular, sexual eukaryotes. Perhaps this difference resides in the particular MCTs that evolved in the former groups and not in red algae. One is left to wonder then: if red algae were the only existing complex multicellular clade, would any of their features be considered a MET, or just a curious anomaly?

On the other hand, a number of proposed METs appear to have happened only once. In the correlated triumvirate of eukaryote, sexual reproduction and multicellularity METs, only the last one appears to have evolved more than once. Similarly, nothing akin to humans appears previously in the historical record. This suggests that all of these were and are particularly unlikely events. As discussed earlier, the evolution of eukaryotes from prokaryotes might have required a particular series of fortuitous and concurrent biological innovations and catalysts, unlikely to ever be repeated. Finally, as regards the transition to humans – why has it happened only once, and taken four billion years to evolve a large-brained species capable of producing and using Level IV and V information? This remains a question to be answered.

In closing, major transitions are critical points of exploration to understand life's history on Earth. We posit that two types of major transitions occur within species or clades: METs and MCTs. The former represents Fusions and/or Information Leaps, whereas the latter includes critical morphological innovations. Some METs and MCTs either by themselves or in concert with each other, have consequences that ripple across the globe as MSTs, altering ecosystems at large and possibly irreversible scales. The process of categorizing events into this new framework highlights the importance of facilitation and biotic and abiotic catalysts in driving ecosystem transformations. Furthermore, levels of information, from the origination of genomes to written language, and possibly machine-produced dark information, are intrinsic to facilitating major events. An interactive scheme encompassing evolutionary fusions, information leaps and morphological innovations with ecosystem changes, paints a broader and potentially more accurate picture of life's past on Earth and provides glimpses into the future.

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

PN initially conceived and organized the project. ANR, KKD, ESHL, and PN led in editing and preparing the manuscript. All authors contributed to researching and writing on one or more sections.

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Life History and the Transitions to Eusociality in the Hymenoptera

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Although indirect selection through relatives (kin selection) can explain the evolution of effectively sterile offspring that act as helpers at the nest (eusociality) in the ants, bees, and stinging wasps (aculeate Hymenoptera), the genetic, ecological, and life history conditions that favor transitions to eusociality are poorly understood. In this study, ancestral state reconstruction on recently published phylogenies was used to identify the independent transitions to eusociality in each of the taxonomic families that exhibit eusociality. Semisociality, in which a single nest co-foundress monopolizes reproduction, often precedes eusociality outside the vespid wasps. Such a route to eusociality, which is consistent with groups consisting of a mother and her daughters (subsocial) at some stage and ancestral monogamy, is favored by the haplodiploid genetic sex determination of the Hymenoptera (diploid females and haploid males) and thus may explain why eusociality is common in the Hymenoptera. Ancestral states were also reconstructed for life history characters that have been implicated in the origins of eusociality. A loss of larval diapause during unfavorable seasons or conditions precedes, or coincides with, all but one transition to eusociality. This pattern is confirmed using phylogenetic tests of associations between state transition rates for sweat bees and apid bees. A loss of larval diapause may simply reflect the subsocial route to eusociality since subsociality is defined as females interacting with their adult daughters. A loss of larval diapause and a gain of subsociality may be associated with an extended breeding season that permits the production of at least two broods, which is necessary for helpers to evolve. Adult diapause may also lower the selective barrier to a first-brood daughter becoming a helper. Obligate eusociality meets the definition of a major evolutionary transition, and such transitions have occurred five times in the Hymenoptera.

Keywords: eusociality, life history, diapause, bees, wasps, major evolutionary transition

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INTRODUCTION

The evolution of an effectively sterile worker caste, known as eusociality (see **Table 1** for definitions of social structures), has occurred many times in the aculeate Hymenoptera (ants, bees, and stinging wasps), but explaining these transitions remains a challenge. Although sterile workers are understood to evolve through kin selection (Hamilton, 1964a,b; see **Table 2** for definitions of key life history terms), the genetic, ecological, and life history conditions favoring reproductive altruism remain poorly understood (Bourke, 2019; Field and Toyoizumi, 2020). At a minimum, a nest-founding female must live long enough in an environment with a sufficiently long breeding season

TABLE 1 | Definitions of sociality.

Broad	Narrow
Presocial — Females nest alone	*Solitary* — Females nest alone and mass provision their nests. They do not interact with their developing young.
	Subsocial — Females nest alone but interact with their developing larvae. Females may overlap with their adult daughters.
Parasocial — Females of the same generation interact on the same nest.	*Communal* — Each female builds, oviposits in, and provisions her own cells.
	Quasisocial — All females cooperate in building and provisioning brood cells, and all females oviposit.
	Semisocial — Some females lay most or all of the eggs. Other females, with limited egg-laying opportunities, are relegated to foraging, nest building, and caring for the young.
Eusocial — Multiple females cooperate in nesting and exhibit reproductive division of labor and there is an overlap of generations so that adult offspring assist their mother.	*Primitively eusocial* — Colonies are small and short-lived, and morphological differences between reproductive and non-reproductive females are minimal or non-existent.
	Advanced eusocial — Colonies are large and complex and often long-lived. Reproductive castes are often morphologically distinct from non-reproductive castes.

TABLE 2 | Definitions of key life history terms.

Term	Definition
Brood	A female's set of offspring of the same age
Brood provisioning — mass	All the food required to complete development is provided at the egg or early larva stage
Brood provisioning — progressive	A larva is fed throughout its development
Diapause	A period of developmental dormancy during an unfavorable season or period
Foundress	An adult female that establishes a nest
Haplodiploidy	Genetic sex determination in which females are diploid (two genome copies) and males are haploid (one genome copy)
Kin selection	Natural selection operating through interactions with genetic relatives
Monogamy	A single female mates with a single male
Monogyny	A single reproductive female in a nest
Monogyny — functional	A single nest co-foundress is responsible for most or all the reproduction in a nest
Prepupa	A last instar larva
Queen	A mated, reproductive female; usually reserved for eusocial species
Reproductive quiescence	Cessation of reproduction without diapause
Swarm	One or more foundresses (queens) and multiple workers that establish a nest
Worker	A non-mated, effectively sterile female that conducts most nest activities other than reproduction — usually reserved for eusocial species

to produce at least two broods, giving first-brood daughters the opportunity to help raise second-brood siblings. This is known as the subsocial route to eusociality (Wheeler, 1923; Alexander et al., 1991; Bourke, 2011). In addition, it is understood that because monogamy is ancestral to eusocial lineages, a daughter is as related to her own offspring as she is to her siblings (Hughes et al., 2008), whereas a mother remains more closely related to her own offspring than to her daughter's offspring. This relatedness asymmetry means that a mother may be selected to coerce her first-brood daughters to help raise their second-brood siblings and that daughters may offer little or no resistance (Stubblefield and Charnov, 1986; Boomsma, 2009). Thus, the barriers to a first-brood daughter's independent breeding, or the advantages to helping, do not have to be great in order for a worker caste to evolve, as long as a daughter can help raise a sufficient number of siblings. The question then is, what genetic, ecological, and life history conditions favor a daughter foregoing her own reproduction to help her mother?

Numerous traits, including genetic, morphological, behavioral, demographic, life history, and ecological traits, have been hypothesized to favor eusociality, but without any consensus as to which are sufficient and which are necessary (reviewed by Andersson, 1984; Crespi, 1996). For example, whether haplodiploidy favors eusociality remains disputed (Hamilton, 1964b; Trivers and Hare, 1976; Seger, 1983; Fromhage and Kokko, 2011; Gardner et al., 2012; Gardner and Ross, 2013). Considering life history traits, multiple nest foundresses, underground nests, progressive brood provisioning, and breeding aseasonality have each been proposed to select for a long lifespan in a nest foundress, resulting in the required overlap between a foundress and her offspring (Cowan, 1991; Matthews, 1991; Queller, 1996b; Brockmann, 1997; Keller and Genoud, 1997; O'Neill, 2001; Danforth et al., 2019; da Silva, in press). It has also been suggested that aboveground nests favor eusociality because they provide greater opportunities to expand the nest and thus allow greater capitalization of workers' efforts or require the help of workers for the nest's defense (Alexander et al., 1991; Matthews, 1991). A recent phylogenetic comparative study reports a strong association between a loss of larval diapause and the evolution of social living in bees (Santos et al., 2019), and argues that this pattern supports the hypothesis that eusociality is favored by "split sex ratios" between broods that may arise in partially bivoltine species with mated adult diapause (Seger, 1983).

In the present study, information was collected from the literature for life history characters that have been implicated in the evolution of eusociality and for which data are commonly available across the Aculeata. Using recently published phylogenies, ancestral character states were reconstructed to describe the evolutionary histories of life history characters in relation to transitions to eusociality in all four taxonomic families that exhibit eusociality: Vespidae (vespid wasps), Pemphredonidae (aphid wasps), Halictidae (sweat bees), and Apidae (apid bees). This, the first analysis of its kind across the aculeate Hymenoptera, is possible because of new data on the sociality and life histories of poorly studied taxa and because of the recent stabilization of Hymenopteran phylogeny (Branstetter et al., 2017; Peters et al., 2017; Piekarski et al., 2018;

Sann et al., 2018; Bossert et al., 2019). Eusociality was found to have originated more often than previously thought in the Apidae and Halictidae and to be preceded by semisociality outside the Vespidae. A semisocial route to eusociality is consistent with a subsocial route and with ancestral functional monogyny and, because semisociality is favored by haplodiploidy, may explain why eusociality is common in the Hymenoptera. A loss of larval diapause precedes, or coincides with, all but one of the transitions to eusociality. This may simply reflect that subsociality is defined as the interaction between a mother and her adult offspring. In addition, a loss of larval diapause and a gain of subsociality may be associated with an extended breeding season, permitting the production of two broods. Obligate eusociality, which meets the definition of a major evolutionary transition, has evolved five times in the Hymenoptera. An important consequence of these transitions is that inclusive fitness can no longer be calculated for individuals within obligately eusocial groups but must be calculated for the new level of individuality, the colony.

MATERIALS AND METHODS

Data

All available data were collected for sociality and other life history characters for species in taxonomic families exhibiting eusociality: Vespidae (vespid wasps), Pemphredonidae (aphid wasps), Halictidae (sweat bees), and Apidae (apid bees). The life history characters are:

(1) Sociality (solitary, subsocial, communal, quasisocial, semisocial, primitively eusocial, advanced eusocial)
(2) Number of nest foundresses (single, multiple, swarm)
(3) Nest type (ground, stem/wood, cavity, aerial)
(4) Brood provisioning (mass, progressive)
(5) Breeding seasonality (seasonal, aseasonal)
(6) Diapause (prepupal, adult, none, reproductive quiescence)

The definitions of social organizations of Hymenoptera are those of Cowan (1991), which are modified from those of earlier workers (Wilson, 1971; Michener, 1974; Eickwort, 1981; **Table 1**). The highest level of social structure reported for a species was used. Life history characters are defined in **Table 2**. The category of the number of nest foundresses assigned to a species is the highest category recorded. Swarming refers to one or more foundresses and multiple workers establishing a nest. Nests may be excavated in the ground, produced by boring in the soft pith of stems or in soft wood, built in available cavities or constructed in exposed areas. Brood provisioning is either mass, in which a female provides the food required by a larva to complete development all at once, or progressive, in which food is provided incrementally to the growing larva. Diapause refers to a period of developmental dormancy. Prepupal diapause occurs during the last larval instar. Reproductive quiescence refers to a cessation in reproduction without diapause. These data were collected for 317 species and are available as a supplementary dataset for each family (**Supplementary Data**).

Statistical Analyses

All statistical tests and inferences were carried out using the *R* language and environment for statistical computing and graphics (R Core Team, 2019).

Ancestral State Reconstruction

Discrete ancestral character states were reconstructed on recent phylogenies using maximum likelihood to fit hidden Markov models (Beaulieu et al., 2013; Beaulieu and O'Meara, 2014), as implemented in the R package *corHMM* (version 2.1). This method allows rates of transitions between character states to vary across a phylogeny as different rate classes. All transition rates between character states were allowed to differ. Transitions between states could be constrained to not permit certain transitions. The state of the root node was fixed to the ancestral state when there was strong supporting evidence, otherwise the method of FitzJohn et al. (2009) was used to infer the root state (root.p = "maddfitz"). This method weights each root state according to its probability of giving rise to the extant data, given the model parameters and the phylogeny. The states of other internal nodes could also be fixed to test hypotheses about character evolution. Marginal reconstruction was used to estimate the empirical Bayesian posterior probability of each state at each internal node. The state with the highest probability was assigned to each node. One hundred random restarts were used to ensure that the maximum likelihood solution was found. Models with different numbers of rate classes were compared using the Akaike information criterion (AIC). The smallest number of rate classes giving the smallest AIC was used (a difference of 2 AIC units is considered significant). A single rate class is used unless stated otherwise.

For each taxonomic family, the states of the root node for each character were fixed as solitary living, a single nest foundress, ground nesting, mass brood provisioning, seasonal breeding, and prepupal diapause (Wilson, 1971; Michener, 1974; Cowan, 1991; Matthews, 1991; Hunt, 2007; Danforth et al., 2019; Santos et al., 2019), unless stated otherwise. For sociality, state transitions were constrained to follow the subsocial route to eusociality (Alexander et al., 1991; Bourke, 2011; **Figure 1A**). This may occur directly from subsociality to primitive eusociality or indirectly via parasociality. All forms of parasocial living appear to be also subsocial (Lin and Michener, 1972; Alexander et al., 1991; Cowan, 1991). It is assumed that semisocial living evolved from less complex forms of parasociality, in which mutual tolerance and reproductive cooperation evolved (Cowan, 1991). Of the parasocial social structures, only semisociality is assumed to precede primitive eusociality since monogamy is ancestral to eusociality in the Hymenoptera, and this may be functional in the sense that one co-foundress does all or most of the egg laying (Hughes et al., 2008). It is also assumed that primitive eusociality precedes advanced eusociality (Wilson, 1971; Michener, 1974) and that advanced eusociality represents a point of no return, from which there are no reversions to other forms of sociality (Wilson and Hölldobler, 2005; Boomsma and Gawne, 2018). For diapause, transitions were constrained to be sequential (**Figure 1B**), reflecting a reduction in diapause as species adapt incrementally to reduced breeding seasonality

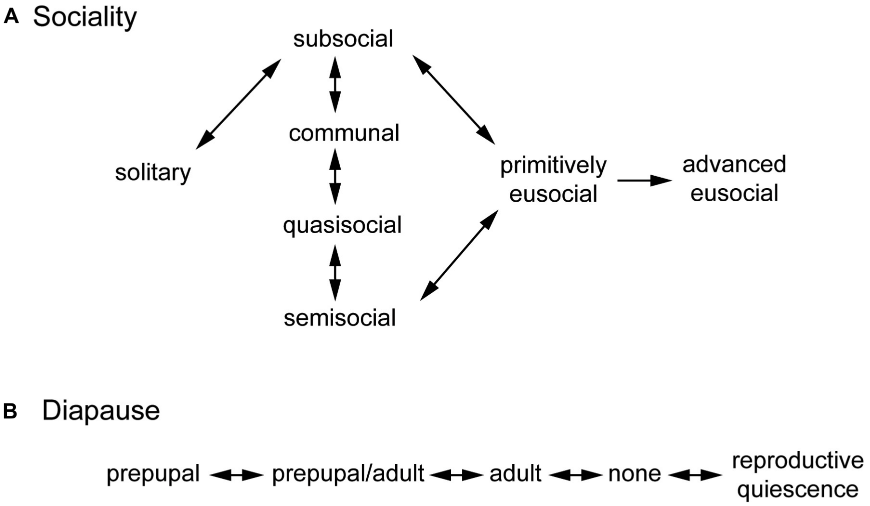


FIGURE 1 | Character state transition models for **(A)** sociality and **(B)** diapause.

(e.g., Saito et al., 2009). Reproductive quiescence represents a non-diapause adaptation to breeding seasonality. This model is supported by examples of sequential pairs of states, but no non-sequential pairs, observed within the same genus (e.g., adult diapause ↔ no diapause within the same genus in the Polistinae and the Stenogastrinae).

Testing the Phylogenetic Dependence of Eusociality on Life History Characters

Whether a transition from non-eusociality to eusociality is dependent on the state of a life history character was tested using Pagel's method of detecting correlated evolution between binary characters on phylogenies (Pagel, 1994). The method uses maximum likelihood to fit models of character state transition rates for each character evolving independently and for the two characters evolving in a correlated fashion, and then compares these models using the log likelihood ratio test to determine if the correlated model provides a better fit. Tests were carried out with sociality as the dependent variable, and *P*-values were corrected for multiple comparisons within each family using the Holm method (Holm, 1979). Analyses were conducted using the function *fitPagel* (method = "fitMk") in the R package *phytools* (version 0.7-70) (Revell, 2012). Sociality and each life history character were converted to binary characters. For example, the states of sociality were converted to non-eusocial and eusocial. The state of the root of each phylogeny was specified for each character as the ancestral states non-eusocial, solitary foundress, ground nesting, mass brood provisioning, seasonal breeding, and prepupal diapause (or diapause, depending on the form of the binary character).

Phylogenies

Vespidae

The phylogeny for the family Vespidae (vespid wasps) is based on nucleotide sequence data from 378 loci across 136 species (Piekarski et al., 2018). Branch lengths represent the amount of evolutionary change. Species for which life history data were collected, but which were not included in the published tree, were added. When the tree included a single species of the same genus, or a closely related genus, a species was added to the terminal node of the included species and given a terminal branch length of zero. Otherwise, species were added to the deepest node of the smallest clade to which they belong (most recent common ancestor, MRCA), and their terminal branch given a length of zero. In some cases, these additions created polytomies. Other phylogenies were used as guides in the placement of species in the following genera: *Vespula* (Lopez-Osorio et al., 2015); *Vespa* (Perrard et al., 2013; Lopez-Osorio et al., 2015); *Polistes* (Santos et al., 2015).

Pemphredonidae

The phylogeny for the family Pemphredonidae (aphid wasps) is extracted from a time-calibrated phylogeny of the superfamily Apoidea (bees and digger wasps) based on target DNA enrichment and transcriptomic sequence data from 195 single-copy protein-coding genes for 174 species (Sann et al., 2018). Species for which life history data were collected were added to the tree as for the Vespidae, except their terminal branches were extended to the present. The placements of added species were based on their taxonomic classification.

Halictidae

The phylogeny for the family Halictidae (sweat bees) is a composite of two time-calibrated phylogenies. One phylogeny comprises the subfamily Halictinae and is based on sequences from three nuclear genes (elongation factor-1 alpha, wingless, and long-wavelength rhodopsin) and one mitochondrial gene (cytochrome c oxidase 1) for 206 species (Gibbs et al., 2012). The placements of the subfamilies Nomioidinae, Nomiinae, and Rophitinae is from a second phylogeny, of bees (clade Anthophila), based on sequences from two nuclear ribosomal genes (18S and 28S) and five nuclear protein-coding genes (elongation factor-1 alpha, wingless, opsin, pol II, and Nak) for 152 species (Cardinal and Danforth, 2013). The ages for the relevant overlapping nodes from the two phylogenies are consistent. Species for which life history data were collected were added, as for the Vespidae, except their terminal branches were extended to the present. Other phylogenies were used in the placement of species in the following groups: Rophitinae (Patiny et al., 2008); Nomiinae (Brady et al., 2006; Patiny et al., 2008); Augochlorini (Gonçalves, 2016); *Agapostemon* (Janjic and Packer, 2003); *Sphecodes* (Habermannova et al., 2013).

Apidae

The phylogeny for the family Apidae (apid bees) is based on genome, transcriptome and ultraconserved element sequences for 79 species (Bossert et al., 2019). Branch lengths represent the amount of evolutionary change. Species for which life history data were collected were added as for the Vespidae. Other phylogenies were used in the placement of species in the following groups: Anthophorinae (Dubitzky, 2007); Nomadinae (Litman et al., 2013); Xylocopinae (Rehan et al., 2012); *Eucera* (Dorchin et al., 2018); Centridini (Martins and Melo, 2016); Meliponini (Rasmussen and Cameron, 2007); *Bombus* (Cameron et al., 2007).

RESULTS

Results for ancestral character state reconstructions are presented for each family, with the origins of eusociality inferred first, followed by the evolutionary histories of life history characters in relation to the origins of eusociality. Phylogenies depicting ancestral state reconstructions for each life history character are given in **Supplementary Figures 1–5**. These results are summarized in **Figure 2**. Results of tests for the dependence of eusociality on each life history character are then presented.

Vespidae

Sociality

Solitary living at the root node is consistent with the solitary parasitoid family Rhopalosomatidae as the extant sister group to the Vespidae (Branstetter et al., 2017). Ancestral state reconstruction infers that primitive eusociality arose independently in the MRCAs of the subfamilies Vespinae + Polistinae and Stenogastrinae (**Figure 3**). These origins descend from nodes inferred to be solitary, but would have passed through subsocial stages, as constrained by the model. This assumption is supported by the subsocial subfamily Zethinae as the sister group to the Vespinae + Polistinae. Advanced eusociality evolved once, in the MRCA of the Vespinae.

Although the swarm-founding Polistinae (Jeanne, 1991) were classified as primitively eusocial because many species do not exhibit discrete caste-specific difference in morphology, size and behavior and workers may become queens in some species (Strassmann et al., 2002; Noll and Wenzel, 2008), they could be considered as advanced eusocial because of their dependence on workers to establish new nests and because of caste-specific differences in some species. The dataset contains two swarm-founding species, *Synoeca septentrionalis* and *Protopolybia exigua*, both in the tribe Epiponini, which forms the sister group to the primitively eusocial Polistini (*Polistes*) (**Figure 3**). Reclassifying these species as advanced eusocial changes their MRCA from primitively eusocial to advanced eusocial but does not affect the state of any other node. This change also does not affect any associations with other life history characters, for which transitions from the root state precede the origin of swarming.

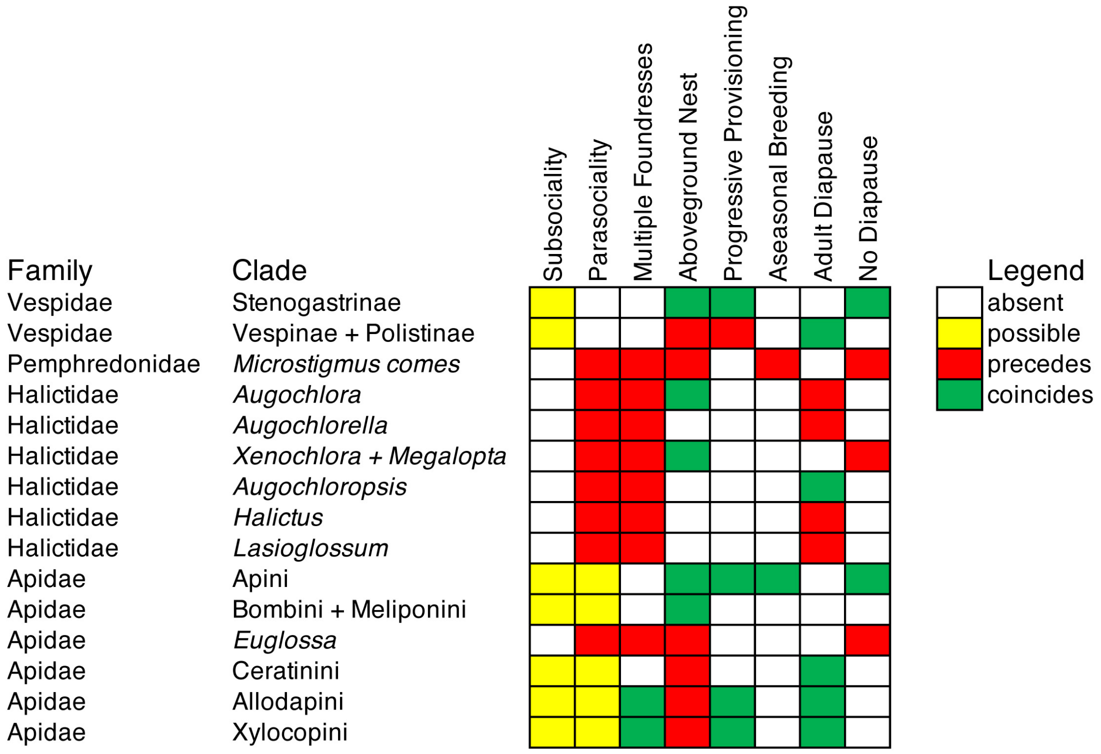
Life History

Both nodes at the origins of primitive eusociality maintain the ancestral state of a single nest foundress (**Figure 3**). Some nodes within the primitively eusocial Polistinae and Stenogastrinae are inferred to have multiple foundresses. Ground nesting was assumed to be ancestral for the Vespidae, although as the extant sister group to the Vespidae are the parasitoid Rhopalosomatidae, there is no direct supporting evidence. However, inferring the state of the root from the data supports ground nesting. The origin of primitive eusociality in the Stenogastrinae coincides with a transition to aerial nesting. However, for the origin of primitive eusociality in Vespinae + Polistinae, the node state is cavity nesting, which is retained from an earlier transition from ground nesting at the node for the MRCA of the Eumeninae + [Zethinae + (Vespinae + Polistinae)]. The primitively eusocial Polistinae have an MRCA with aerial nesting. A transition from mass brood provisioning to progressive provisioning coincides with the origin of primitive eusociality in the Stenogastrainae. Another transition to progressive provisioning occurs in the MRCA of the Zethinae + (Vespinae + Polistinae), thus preceding the origin of primitive eusociality in the Vespinae + Polistinae. The other progressive provisioners are the subsocial genera *Paraleptomenes*, *Montezumia,* and *Abispa* in the Eumeninae. There are no transitions from ancestral seasonal breeding that coincide with or precede the origins of eusociality, although most of the Stenogastrinae live in aseasonal environments. A reversion from primitive eusociality to subsociality in the Stenogastrinae coincides with a reversion from an aseasonal environment to a seasonal environment. Ancestral prepupal diapause is consistent with prepupal diapause in the Rhopalosomatidae (Gurney, 1953). A transition to adult diapause from prepupal diapause coincides with the origin of primitive eusociality in the Vespinae + Polistinae. A transition to no diapause from prepupal diapause coincides with the origin of primitive eusociality in the Stenogastrinae. A reversion from primitive eusociality to subsociality in the Stenogastrinae coincides with a reversion from no diapause to adult diapause, consistent with the associated reversion from an aseasonal environment to a seasonal one.

Pemphredonidae

Sociality

The state of the root was fixed as solitary living, consistent with solitary living in the subtribe Pemphredonina, which forms part of the sister group to the subtribe Spilomenina (**Figure 4**). The Spilomenina contain the only primitively eusocial species in the Pemphredonidae, *Microstigmus comes*. The other members of the Spilomenina, including the congener *Microstigmus*



Family	Clade	Subsociality	Parasociality	Multiple Foundresses	Aboveground Nest	Progressive Provisioning	Aseasonal Breeding	Adult Diapause	No Diapause
Vespidae	Stenogastrinae	🟨	⬜	⬜	🟩	🟩	⬜	⬜	🟩
Vespidae	Vespinae + Polistinae	🟨	⬜	⬜	🟥	🟥	⬜	🟩	⬜
Pemphredonidae	*Microstigmus comes*	⬜	🟥	🟥	🟥	⬜	🟥	⬜	🟥
Halictidae	*Augochlora*	⬜	🟥	🟥	🟩	⬜	⬜	🟥	⬜
Halictidae	*Augochlorella*	⬜	🟥	🟥	⬜	⬜	⬜	🟥	⬜
Halictidae	*Xenochlora + Megalopta*	⬜	🟥	🟥	🟩	⬜	⬜	⬜	🟥
Halictidae	*Augochloropsis*	⬜	🟥	🟥	⬜	⬜	⬜	🟩	⬜
Halictidae	*Halictus*	⬜	🟥	🟥	⬜	⬜	⬜	🟥	⬜
Halictidae	*Lasioglossum*	⬜	🟥	🟥	⬜	⬜	⬜	🟥	⬜
Apidae	Apini	🟨	🟨	⬜	🟩	🟩	🟩	⬜	🟩
Apidae	Bombini + Meliponini	🟨	🟨	⬜	🟩	⬜	⬜	⬜	⬜
Apidae	*Euglossa*	⬜	🟥	🟥	🟥	⬜	⬜	⬜	🟥
Apidae	Ceratinini	🟨	🟨	⬜	🟥	⬜	⬜	🟩	⬜
Apidae	Allodapini	🟨	🟨	🟩	🟥	🟩	⬜	🟩	⬜
Apidae	Xylocopini	🟨	🟨	🟩	🟥	🟩	⬜	🟩	⬜

Legend
⬜ absent
🟨 possible
🟥 precedes
🟩 coincides

FIGURE 2 | Summary of life history character state transitions in relation to transitions to eusociality. Derived life history character states are shown. An independent transition to eusociality occurred at the MRCA of each clade.

nigrophthalmus, are quasisocial. A transition from solitary living to quasisociality occurs in the MRCA of the Spilomenina. Therefore, the transition to primitive eusociality in *M. comes* was preceded by quasisociality and unobserved semisociality.

Life History

A transition to multiple foundresses coincides with the transition to quasisociality in Spilomenina, and therefore multiple founding preceded the origin of primitive eusociality (**Figure 4**). The stem/wood nesting of the Pemphredonina + Stigmina is supported as ancestral when the root state is inferred from the data. A transition to aerial nesting occurs at the MRCA of the Spilomenina and therefore precedes the origin of primitive eusociality. All the members of the Pemphredonina + Stigmina in this dataset are mass provisioners, supporting ancestral mass provisioning. *Microstigmus comes* is a mass provisioner, whereas its congener, *M. nigrophthalmus*, is a progressive provisioner. The other two species in the Spilomenina included in the tree are also progressive provisioners. Most internal nodes are inferred to be mass or progressive provisioning with equal probabilities. Therefore, although progressive provisioning is common in the Spilomenina, the only primitively eusocial species is a mass provisioner. A transition from ancestral seasonal breeding to aseasonal breeding occurs in the MRCA of the Spilomenina and therefore precedes the origin of primitive eusociality. A transition from ancestral prepupal diapause to no diapause occurs in the MRCA of the Spilomenina and therefore also precedes the origin of primitive eusociality.

Halictidae

Sociality

The root node for the Halictidae was fixed as solitary living, consistent with solitary living in the subfamily Rophitinae, the sister group to the remaining Halictidae [Halictinae + (Nomiinae + Nomioidinae)]. A model with two rate classes provides the best fit to the data (AIC: one class 498.97; two classes 413.35; three classes 428.56). A transition from solitary living to communal living occurs at the MRCA of the Halictinae + (Nomiinae + Nomioidinae) (**Figure 5**). A subsequent transition to semisociality occurs at the MRCA of the Augochlorini, followed by independent transitions to primitive eusociality in *Augochlora*, *Augochlorella*, *Xenochlora* + *Megalopta,* and *Augochloropsis*. The state of the MRCA of the Halictini is communal living and there are independent transitions to semisociality in the Hemihalictus series of the genus *Lasioglossum* and the MRCA of *Halicuts* + *Thrincohalictus*. Primitive eusociality then evolves in the MRCA of *Halictus* and several times in the Hemihalictus series of *Lasioglossum*.

Previous studies have proposed either two origins (Danforth, 2002; Brady et al., 2006), or a single origin (Gibbs et al., 2012), of primitive eusociality in the Halictini. To test the hypothesis of a single origin, the node for the MRCA of Halictini was fixed as primitively eusocial, but this model gave a marginally

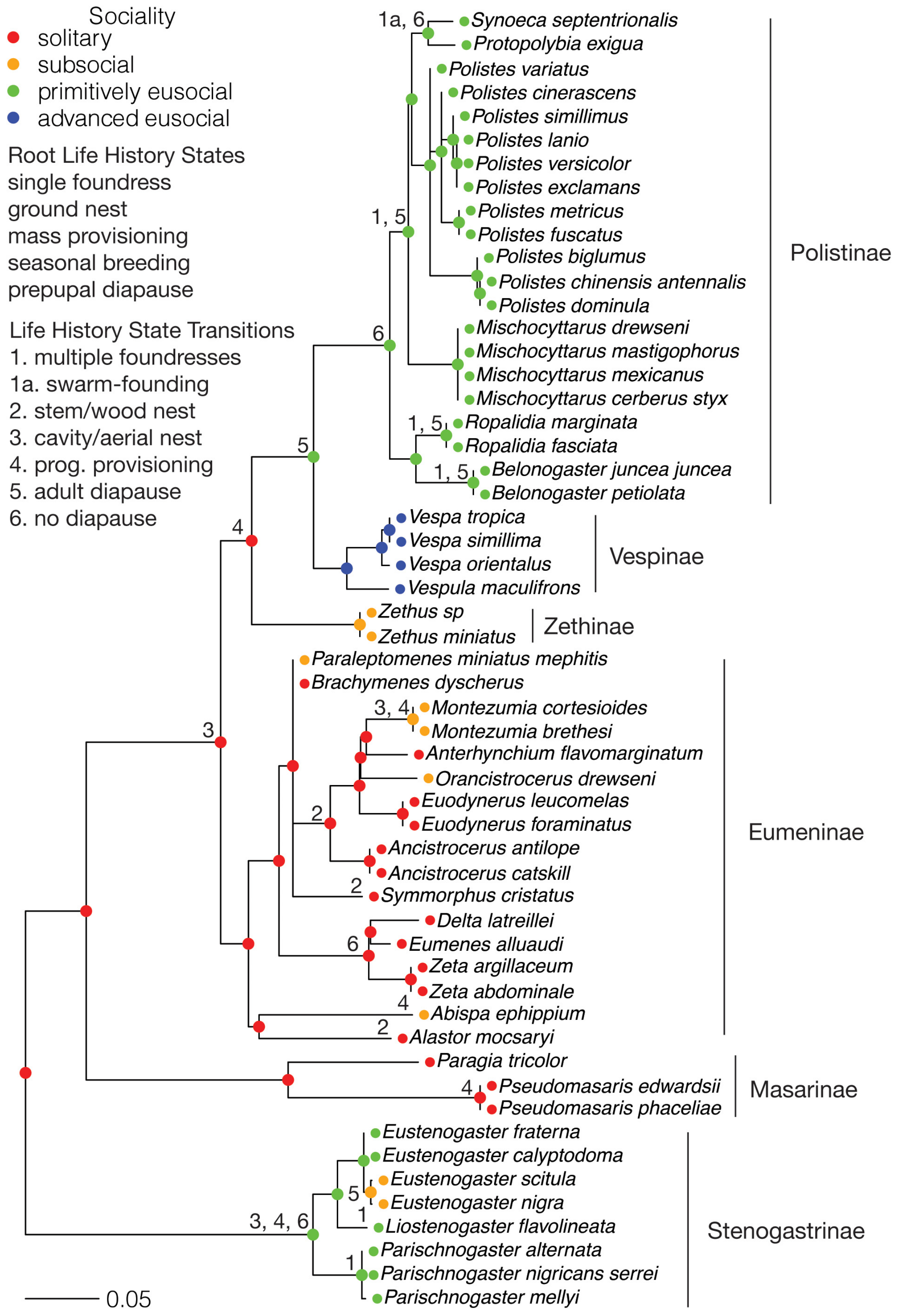


FIGURE 3 | Vespidae: Ancestral states for sociality and transitions from the root state for each life history character. Subfamilies are indicated. The scale bar is in units of substitutions per site.

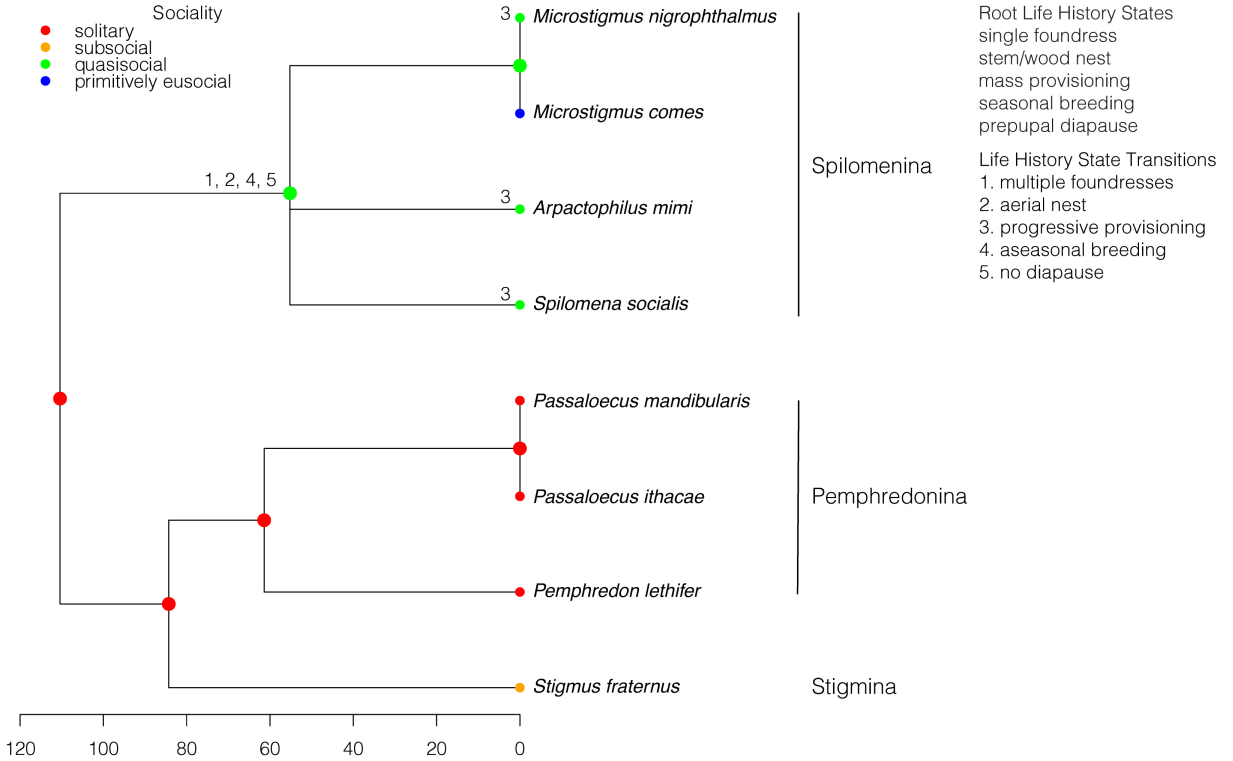


FIGURE 4 | Pemphredonidae: Ancestral states for sociality and transitions from the root state for each life history character. Subtribes are indicated. The scale is in millions of years.

poorer fit to the data than fixing the node as communal (AIC 415.87 vs. 414.98). Previous studies of the Augochlorini, based on limited data on social behavior, have proposed a single origin of eusociality within the clade and possibly one reversion to solitary living (Danforth and Eickwort, 1997; Danforth, 2002; Brady et al., 2006). The addition of newer data for this group suggests at least four independent origins of eusociality within the Augochlorini. The number of reversions from primitive eusociality within the clade cannot be determined because of polytomies.

The two rate classes (rates scaled to a maximum of 100) in the preferred model for the Halictidae correspond to low transition rates between semisociality (sem) and primitive eusociality (prim) in the Halictini ($q_{sem \rightarrow prim} = 0.13$; $q_{prim \rightarrow sem} < 0.01$) and high rates in the Augochlorini ($q_{sem \rightarrow prim} = 60.99$; $q_{prim \rightarrow sem} = 100$).

Life History

The number of foundresses could not be reconstructed accurately; most nodes were inferred to have nearly equal probabilities of single and multiple foundresses, suggesting that this character is very labile. Nevertheless, a transition from ancestral single founding to multiple founding is inferred for the MRCA of the subfamily Halictinae, preceding the origins of eusociality in the subfamily (**Figure 5**). Nearly all species are ground nesters. Transitions from ancestral ground nesting to stem/wood nesting occur in some lineages of the Augochlorini that evolve eusociality: *Augochlora* and *Xenochlora* + *Megalopta*. All species are mass provisioners. Nearly all species have seasonal breeding. The root node was fixed as prepupal diapause, consistent with prepupal diapause in the Rophitinae. A transition from ancestral prepupal diapause to adult diapause is inferred for the MRCA of the Halictinae, thus preceding the origins of eusociality in the subfamily. However, there is a transition to no diapause in the MRCA of the Augochlorini followed by reversions to adult diapause in the MRCAs of *Augochlora* + *Augochlorella* and *Augochloropsis* and a transition to reproductive quiescence in the MRCA of the clade *Caenaugochlora* + (*Xenochlora* + *Megalopta*), all of which contain lineages that evolved eusociality.

Apidae

The subfamilies Apinae and Xylocopinae were analyzed separately since eusociality appears to have evolved independently in them and advanced eusociality is exclusive to the Apinae (Danforth et al., 2013). In addition, transition rates, including reversions, between character states may be significantly different between the two subfamilies. Frequent gains and losses of primitive eusociality have been inferred for the Xylocopinae (Wcislo and Danforth, 1997; Rehan et al., 2012).

Apinae

Sociality

The root node was fixed as solitary, consistent with the mostly solitary tribe Centridini, the sister group to the

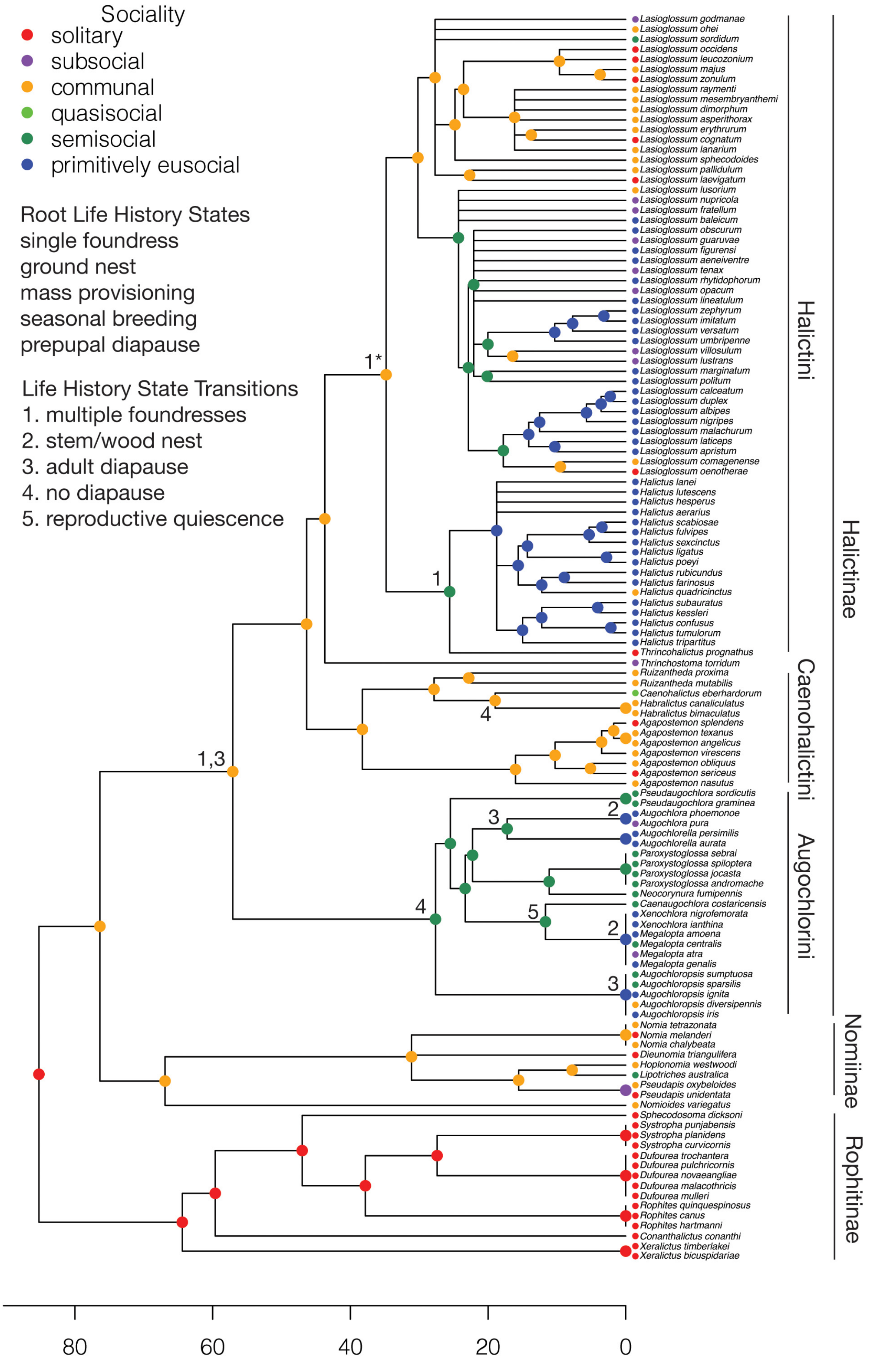


FIGURE 5 | Halictidae: Ancestral states for sociality and transitions from the root state for each life history character. An asterisk indicates a reversion to the root state. Major subfamilies and tribes are indicated. The scale is in millions of years.

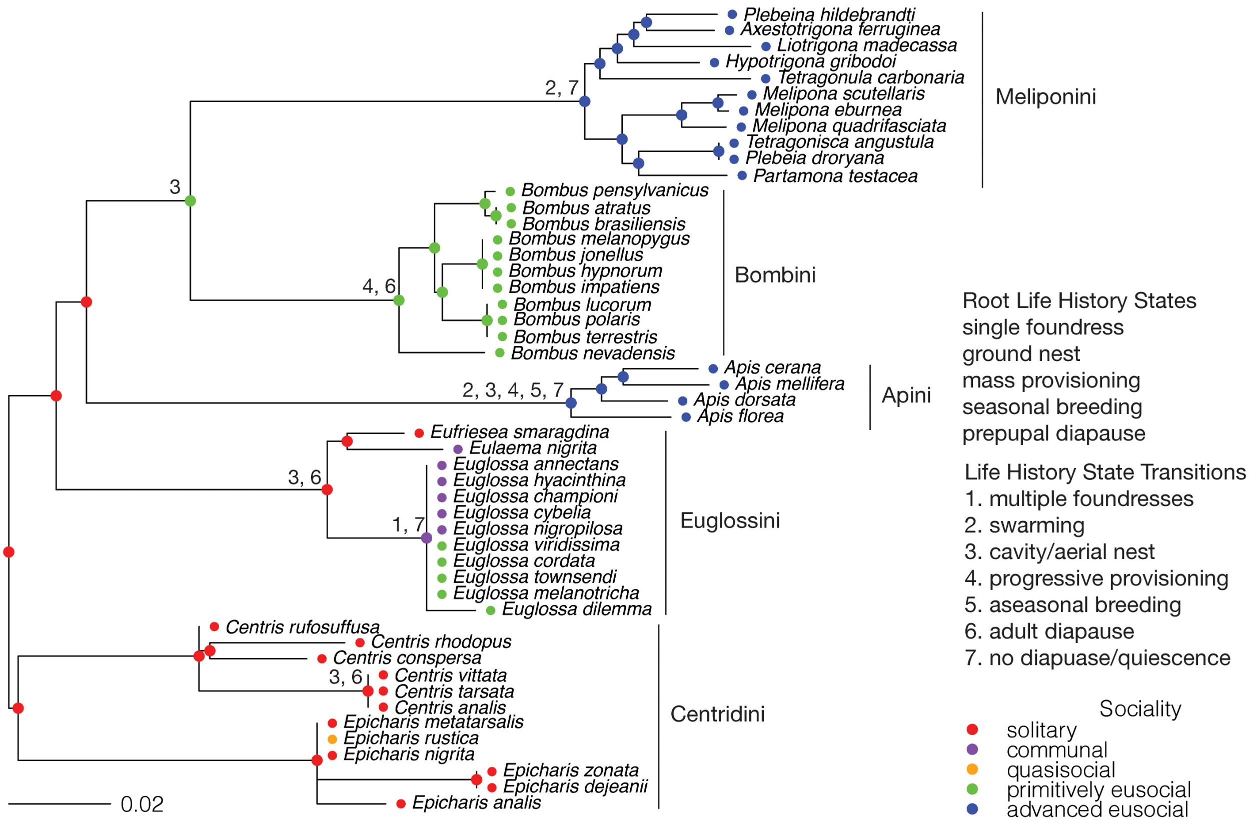


FIGURE 6 | Apinae: Ancestral states for sociality and transitions from the root state for each life history character. Tribes are indicated. The scale bar is in units of substitutions per site.

remaining Apinae. Eusociality is inferred to have evolved at least three times (**Figure 6**). Primitive eusociality evolved at least once in the genus *Euglossa* (tribe Euglossini) from a node with communal living, and therefore through unobserved semisociality. Primitive eusociality also evolved in the MRCA of the tribes Meliponini + Bombini, with advanced eusociality evolving in the MRCA of the Meliponini. This transition to primitive eusociality occurred from a node with solitary living and therefore is assumed to have passed through either subsociality or semisociality. Advanced eusociality also evolved in the MRCA of the tribe Apini. This transition is from a node with solitary living and is therefore assumed to have passed through either subsociality or semisociality and then primitive eusociality. To test whether there is a single origin of eusociality for the exclusively eusocial Apini + (Bombini + Meliponini), the MRCA for this clade was fixed as primitively eusocial, but this model gave a poorer fit to the data than when the node was fixed as solitary (AIC 197.46 vs. 195.05). Eusociality has also been inferred to have evolved in the MRCA of the corbiculate Apinae (Euglossini, Apini, Bombini, and Meliponini) (Cardinal and Danforth, 2011). This hypothesis was tested by fixing the MRCA of the corbiculates as primitively eusocial, but this model also gave a poorer fit to the data than fixing the node as solitary (AIC 201.36 vs. 194.21). Therefore, eusociality evolved twice in the Apini + (Bombini + Meliponini) and advanced eusociality evolved independently in the Meliponini and the Apini.

There have been suggestions that the non-parasitic *Bombus* (bumblebees) be reclassified as advanced eusocial, rather than primitively eusocial as they are classified here, on the basis of various criteria (Crespi and Yanega, 1995; Kocher and Paxton, 2014; Boomsma and Gawne, 2018; Richards, 2019; Holland and Bloch, 2020). This reclassification has the effect of changing the MRCA of the Meliponini + Bombini from primitively eusocial to advanced eusocial. However, the MRCA of the Apini + (Bombini + Meliponini) remains solitary, and therefore the conclusion of two origins of eusociality in the Apini + (Bombini + Meliponini) does not change.

Life History

The state of the root for the number of foundresses was fixed as single, consistent with a solitary ancestor. Separate transitions from solitary founding to swarming coincide with both origins of advanced eusociality, supporting two origins of advanced eusociality (**Figure 6**). The primitively eusocial Bombini mostly retain ancestral single founding. A transition to multiple founding from single founding preceded the origin of primitive eusociality in the Euglossini. The state of the root for nest type was fixed as ground nesting, consistent with the mostly ground nesting Centridini. Cavity and aerial

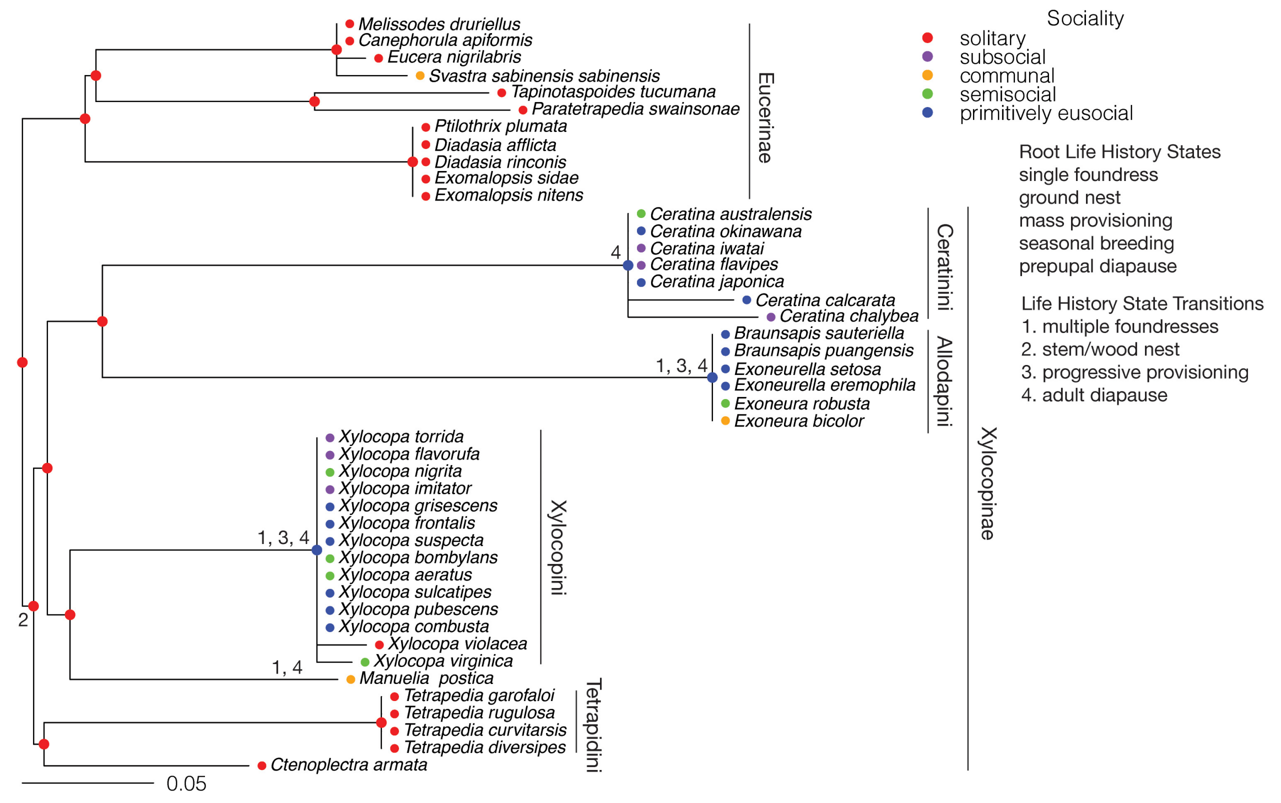


FIGURE 7 | Xylocopinae and Eucerinae: Ancestral states for sociality and transitions from the root state for each life history character. Major tribes of the Xylocopinae are indicated. The scale bar is in units of substitutions per site.

nesting were merged into a single state, cavity/aerial nesting. Separate transitions from ground nesting to cavity/aerial nesting coincide with the two origins of eusociality in the Meliponini + Bombini and Apini. A transition from ground nesting to cavity/aerial nesting occurs at the MRCA of the Euglossini, and thus cavity/aerial nesting precedes the origin of primitive eusociality in *Euglossa*. The state of the root for brood provisioning was fixed as mass provisioning, consistent with mass provisioning in the Centridini. A transition from mass provisioning to progressive provisioning occurs in the MRCA of the Apini, coinciding with this origin of advanced eusociality. A transition from mass provisioning to progressive provisioning also occurs in the MRCA of the primitively eusocial Bombini, while the advanced eusocial Meliponini retain ancestral mass provisioning. Therefore, the Apini and Bombini evolved progressive provisioning independently. The state of the root for breeding seasonality was fixed as seasonal. Most species live in a seasonal environment. A transition from seasonality to aseasonality occurs at the MRCA of the advanced eusocial Apini. The state of the root for diapause was fixed as prepupal, consistent with prepupal diapause being common in the Centridini. A transition from prepupal to no diapause coincides with the origin of advanced eusociality in the MRCA of the Apini. The state of the MRCA of the Meliponini + Bombini is prepupal diapause, which coincides with the origin of primitive eusociality for this clade. There is a transition to adult diapause for the MRCA of the Bombini and reproductive quiescence for the MRCA of the Meliponini. A transition from prepupal to adult diapause occurs in the MRCA of the Euglossini and is followed by a transition to no diapause in *Euglossa*, which thus precedes the evolution of eusociality in this group.

Xylocopinae + Eucerinae

Sociality

The subfamilies Xylocopinae + Eucerinae form the sister group to the Apinae. The state of the root for the Xylocopinae + Eucerinae was fixed as solitary, consistent with the mostly solitary Eucerinae. Transitions from solitary living to primitive eusociality (through subsociality or semisociality) occur for the MRCAs of the tribes Ceratinini, Allodapini, and Xylocopini of the Xylocopinae (**Figure 7**). In each of these tribes there are reversals to presociality and parasociality. Therefore, primitive eusociality evolved independently in the Ceratinini, Allodapini, and Xylocopini. A previous study suggested that sociality, defined broadly to cover all forms of sociality, evolved once in the Xylocopinae (Rehan et al., 2012). To test the hypothesis that eusociality evolved once in the Xylocopinae and was subsequently lost in some lineages, the state of the MRCA of the clade (Ceratinini + Allodapini) + (Xylocopini + Manueliini) was fixed as primitively eusocial, but this gave a poorer fit to the data than fixing the node as solitary (AIC 236.38 vs. 230.17).

Life History

The state of the root for the number of foundresses was fixed as single, consistent with ancestral solitary living. A transition from a single foundress to multiple foundresses coincides with each of the transitions to primitive eusociality in the MRCAs of the Allodapini and Xylocopini (**Figure 7**). The state of the root for nest type was fixed as ground nesting, consistent with ground nesting in the Eucerinae. A transition to stem/wood nesting from ground nesting occurs in the MRCA of the Xylocopinae, and therefore precedes the origins of eusociality within this subfamily. The state of the root for brood provisioning was fixed as mass provisioning, consistent with mass provisioning in the Eucerinae. A transition from mass provisioning to progressive provisioning coincides with each of the transitions to primitive eusociality in the MRCAs of the Allodapini and Xylocopini. All species live in seasonal environments. The state of the root for diapause was fixed as prepupal, consistent with prepupal diapause in the Eucerinae. Transitions to adult diapause from prepupal diapause occur at the MRCAs of the Ceratinini, Allodapini, and Xylocopini. Therefore, transitions to adult diapause coincide with all three transitions to eusociality.

Statistical Tests of the Dependence of Eusociality on Life History

Tests for the dependence of sociality on each life history character, using binary versions of the characters, were carried out for each family except the Pemphredonidae, which contain a single eusocial species. The effect of diapause on the evolution of eusociality was tested in three ways to account for: (1) a loss of prepupal diapause, (2) a loss of diapause altogether, and (3) the effect of adult diapause on its own. After correcting for multiple comparisons within the family, none of the life history characters was significantly associated with sociality in the Vespidae ($P > 0.3$; **Table 3**). For the Halictidae, nest type and form of diapause have highly significant associations with sociality. For nest type, the transition from non-eusociality to primitive eusociality occurs at a much higher rate when nests are in a stem or wood ($q = 8523.124$) than when they are in the ground ($q = 0.002$) ($P = 1.705 \times 10^{-10}$). For diapause, primitive eusociality evolves only after prepupal diapause has been lost ($P = 2.214 \times 10^{-11}$). For the Apidae, nest type, brood provisioning, and diapause are significantly associated with sociality. For nest type, eusociality evolves only when nests are aboveground ($P = 0.0132$), with the effect being due to cavity or aerial nesting ($P = 0.0008$). For brood provisioning, eusociality evolves at a higher rate with progressive provisioning ($q = 29.57$) than with mass provisioning ($q = 1.042$) ($P = 0.0105$). For diapause, eusociality evolves only after the loss of prepupal diapause ($P = 0.0132$), due to transitions to adult diapause ($P = 0.0250$).

TABLE 3 | Tests for the dependence of eusociality on binary life history characters within each family using Pagel's method.

Derived character state	Log-likelihood ratio	*P**
Vespidae		
No. of foundresses: Multiple	4.8584	0.6167
Nest type: Aboveground	0.4312	1.0000
Nest type: Cavity/aerial	1.2984	1.0000
Brood provisioning: Progressive	6.5256	0.3064
Breeding seasonality: Aseasonal	0.6465	1.0000
Diapause: No prepupal	2.8162	1.0000
Diapause: None	0.3238	1.0000
Diapause: Adult vs. prepupal	4.0196	0.8040
Halictidae		
No. of foundresses: Multiple	−2.2129	1.0000
Nest type: Aboveground	48.2006	**1.705×10^{-10}**
Nest type: Cavity/aerial (none)	–	–
Brood provisioning: Progressive (none)	–	–
Breeding seasonality: Aseasonal	0.6982	1.0000
Diapause: No prepupal	52.6485	**2.214×10^{-11}**
Diapause: None	0.8351	1.0000
Diapause: Adult vs. prepupal	7.6870	0.0859
Apidae		
No. of foundresses: Multiple	4.9344	0.2544
Nest type: Aboveground	12.001	**0.0132**
Nest type: Cavity/aerial	17.8728	**0.0008**
Brood provisioning: Progressive	12.9801	**0.0105**
Breeding seasonality: Aseasonal	2.4075	0.5074
Diapause: No prepupal	12.2448	**0.0132**
Diapause: None	2.7435	0.5074
Diapause: Adult vs. prepupal	10.1543	**0.0250**

**P-values are corrected for multiple comparisons within each family using the Holm method; values < 0.05 are indicated in bold font.*

DISCUSSION

Transitions to Eusociality

Eusociality is estimated here to have originated at least 15 times across the aculeate Hymenoptera, not counting a single origin in the ants (**Figure 2**). The two origins of primitive eusociality in the Vespidae, in the Stenogastrinae and the Vespinae + Polistinae, are consistent with previous analyses (Hines et al., 2007; Piekarski et al., 2018). Primitive eusociality is inferred to have arisen at least six times in the Halictinae: at least twice in the Halictini (*Halictus* and the Hemihalictus series of *Lasioglossum*) and four times in the Augochlorini. Previous studies have proposed either two origins (Danforth, 2002; Brady et al., 2006), or a single origin (Gibbs et al., 2012), of primitive eusociality in the Halictini and a single origin of primitive eusociality within the Augochlorini (Danforth and Eickwort, 1997; Danforth, 2002; Brady et al., 2006). The several independent origins of eusociality in the Augochlorini inferred in this study are the result of the addition of more recent data on social behavior. The Augochlorini exhibit much higher rates of transition to and from eusociality than the Halictini. In the Apinae, eusociality is inferred to have evolved three times, twice in the Apini + (Bombini + Meliponini) and at least once in *Euglossa*, with advanced eusociality evolving independently in the Apini and Meliponini. Based on a tree topology that is no longer supported, a previous analysis inferred a single origin of eusociality in the Apinae (Cardinal and Danforth, 2011). Two origins of advanced eusociality in the

Apinae are consistent with previous analyses (Winston and Michener, 1977; Cameron, 1993; Cardinal and Danforth, 2011). In the Xylocopinae, primitive eusociality is inferred to have evolved independently in each of the Ceratinini, Allodapini, and Xylocopini. A previous study proposed a single origin of eusociality for the Allodapini (Schwarz et al., 2006). However, a more recent study proposed that sociality, defined broadly, evolved once in the Xylocopinae and was followed by reversions to non-social living (Rehan et al., 2012). In the present study, the MRCA of the Xylocopinae is inferred to be solitary. This discrepancy may reflect the more recent and accurate data on sociality and phylogeny in the present study. Frequent gains and losses of primitive eusociality had been inferred for the Xylocopinae (Wcislo and Danforth, 1997; Rehan et al., 2012).

Routes to Eusociality and Life History

To summarize the routes to eusociality and the associations of life history character states with transitions to eusociality determined through ancestral state reconstruction, the derived states subsociality, parasociality, multiple founding of nests, aboveground nesting, progressive brood provisioning, aseasonal breeding, adult diapause, and no diapause were each classified as being absent, possibly present but unobserved, preceding or coinciding with respect to each origin of eusociality (**Figure 2**). A state may be possibly present in the case of subsociality or parasociality when a transition occurs from solitary living to eusociality on a phylogeny because it is assumed to have passed through a subsocial or semisocial stage that has not been observed. Parasociality is observed to precede or possibly precede 13 of the 15 origins of eusociality, suggesting that semisociality provides a common route to eusociality. The loss of prepupal diapause, in the form of either adult diapause or no diapause, precedes, or coincides with, all except one origin of eusociality.

A semisocial route to eusociality is consistent with a subsocial route since all semisocial species appear to also be subsocial (Lin and Michener, 1972; Alexander et al., 1991; Cowan, 1991). It is also consistent with eusocial lineages being ancestrally monogamous since monogyny is often functional, involving multiple foundresses but with only one foundress responsible for most or all of the egg laying; functional monogyny is ancestral for eusocial vespid wasps, ants, and corbiculate bees (Hughes et al., 2008). A semisocial route to eusociality has been proposed previously and may remain evident as a semisocial stage in the lifecycle of many primitively eusocial species (Lin and Michener, 1972).

Interestingly, a semisocial stage in the evolution of eusociality could resurrect a causal link between haplodiploidy and eusociality (Hamilton, 1964b). Haplodiploidy is not currently considered to favor eusociality because although it increases the genetic relatedness between full sisters it also reduces the relatedness between sisters and brothers, resulting in no advantage of haplodiploidy when the sex ratio of reproductive siblings is even (Trivers and Hare, 1976). However, subordinate, worker-like co-foundresses in semisocial groups of full sisters may experience indirect fitness benefits by raising nieces and nephews, and these are more closely related to them under haplodiploidy than under diploidy. This argument has been made in passing (Trivers and Hare, 1976; West-Eberhard, 1978; Maynard Smith, 1989, p. 178; Pamilo, 1991; Bourke and Franks, 1995, p. 84) but does not appear to have been developed further. This argument depends on co-foundresses being closely related, which they are in primitively eusocial paper wasps (Strassmann et al., 1989; Ross and Carpenter, 1991), hover wasps (Turillazzi, 2012), sweat bees (Brand and Chapuisat, 2016), carpenter bees (Schwarz, 1987; Hurst et al., 1997; Hogendoorn and Velthuis, 1999), and orchid bees (Andrade et al., 2016; Freiria et al., 2017). The conditions that favor multiple females founding a nest may include higher nest survival (Queller, 1996b; Itô and Kasuya, 2005), higher per capita reproduction (Bartz and Hölldobler, 1982; Schwarz, 1988), assured fitness returns for the dominant co-foundress (Lucas and Field, 2011), or subordinate co-foundresses (Gadagkar, 1990; Shreeves et al., 2003) and the opportunity for nest inheritance by subordinate co-foundresses (Leadbeater et al., 2011).

Another interesting consequence of a semisocial stage in the transition to eusociality is that if semisocial groups have a higher number of offspring per capita, the result of local resource enhancement (Schwarz, 1988), then females may have higher a reproductive value than males, which may select for a female-biased sex ratio (West, 2009). This in turn favors eusociality under haplodiploidy since helpers then help to raise closely related sisters disproportionately. Semisociality may also be a preadaptation to the mutual tolerance and reproductive altruism necessary for primitive eusociality (Lin and Michener, 1972).

The Vespidae are an exception in that there is no evidence of parasociality preceding, or coinciding with, their two origins of eusociality (Piekarski et al., 2018; **Figure 2**), although multiple nest foundresses are common in the paper wasps (Polistinae). The eusocial Vespidae, however, exhibit progressive brood provisioning exclusively, in contrast to several other eusocial clades across the Hymenoptera that are mass provisioners. A possible explanation is that multiple founding and progressive provisioning are alternative strategies for dealing with scarce larval food. When food is scarce, having multiple foundresses may increase the chances of adequately mass provisioning the first brood. Alternatively, a single foundress would have to provision progressively to meet the demands of her first brood. Some solitary eumenine vespid wasps may practice mass provisioning when prey is abundant but shift to progressive or delayed provisioning when prey is scarce (Evans, 1977; Cowan, 1991). This may explain the evolution of progressive provisioning (O'Neill, 2001). Whichever strategy is used, food scarcity would also favor a female-biased first brood if they could help raise a second brood. Both progressive provisioning and multiple founding may also select for an extended foundress lifespan, which could overlap that of her first brood and thus permit eusociality to evolve (da Silva, in press). Progressive provisioning may select for an extended foundress lifespan because it extends parental care. Multiple founding may do so because the subordinate co-foundresses do most of the risky foraging, thereby reducing the extrinsic mortality of the dominant foundress (Metcalf and Whitt, 1977; Strassmann et al., 1984; Garofalo, 1985; Giannotti and Machado, 1994; Shreeves and Field, 2002;

Itô and Kasuya, 2005). Perhaps an impetus for eusociality was scarcity of larval food.

These roles for progressive provisioning and multiple founding may explain clades of eusocial species that are exclusively mass provisioning, but with multiple founding or swarming, such as stingless bees (Meliponini) and some clades of sweat bees (Halictinae), while other clades are exclusively progressive provisioning, but with single founding, such as bumblebees (Bombini), and vespine wasps (Vespinae) (**Figure 2**). Most other eusocial clades contain species that combine progressive provisioning and multiple founding: clades of paper wasps (Polistinae), honeybees (Apini), and some clades of carpenter bees (Xylocopinae). The combination of strategies may be common because multiple founding reduces the dependence of the first brood on a single progressively provisioning female over the long provisioning period (Field, 2005).

Life History Dependencies

A more rigorous assessment of the association of sociality and life history characters involved testing for dependence of the transition from non-eusociality to eusociality on transitions in binary life history characters. These tests were done for each family separately to allow for differences in natural history and life history between families. No significant dependencies were observed for the Vespidae, very likely because there are only two transitions to eusociality in this family. The strongest dependencies were identified in the Halictidae, for aboveground nesting and a loss of prepupal diapause. The Apidae also showed these dependencies in addition to a dependency on progressive brood provisioning. Progressive provisioning is discussed above and the loss of prepupal diapause is discussed below. An aboveground nest may favor eusociality because it provides a greater opportunity to expand the nest and therefore allows greater capitalization of the efforts of workers (Alexander et al., 1991; Matthews, 1991). Alternatively, an aboveground nest may require the help of workers for its defense. However, this is unlikely to be important since nest defense is associated with the origin of workers of both sexes in other eusocial taxa, while the origin of exclusively female workers in the Hymenoptera is explained by their primary role in alloparental care (Ross et al., 2013).

The Loss of Prepupal Diapause

The loss of prepupal diapause precedes, or coincides with, all but one transition to eusociality identified by ancestral state reconstruction and is a statistically significant predictor of transitions to eusociality in the Halictidae and Apidae. The only exception to this pattern is the transition to primitive eusociality in the MRCA of the Bombini + Meliponini, which retains ancestral prepupal diapause (**Figure 2**). Prepupal diapause is, however, subsequently lost in the MRCAs of the Bombini (adult diapause) and Meliponini (reproductive quiescence). This sequence of events may suggest that either the assignment of prepupal diapause to the MRCA of the Bombini + Meliponini is incorrect or that eusociality evolved independently in the Bombini and Meliponini. The latter possibility would explain the very different life histories of these two groups, with the Bombini having single foundresses and progressive provisioning, while the Meliponini exhibit swarming and retain ancestral mass provisioning.

In a recent phylogenetic comparative study, Santos et al. (2019) report that a loss of prepupal diapause is associated with nearly every origin of sociality in bees, with sociality defined as subsociality, parasociality, or eusociality. The present study shows that this applies to most transitions to eusociality specifically. The authors of the previous study argue that this pattern supports the "Seger hypothesis," that eusociality is favored by a female bias in the sex ratio of reproductive offspring in the foundress' second brood or the second generation of offspring, caused by first-brood males mating with both first-brood females and second-brood/generation females before these enter diapause (Seger, 1983). The logic is that because of haplodiploidy, female workers will experience higher indirect fitness by helping to raise a female-biased second brood/generation.

However, there does not appear to be any evidence in support of the Seger hypothesis. In particular, the limited number of cases of sex ratio differences between broods/generations have many other possible explanations (Cowan, 1991; O'Neill, 2001; West, 2009), there is no evidence that males mate across broods/generations (Yanega, 1996), the requirement for mated adult diapause does not explain the evolution of eusociality in carpenter bees (Xylocopinae), which have unmated adult diapause (Danforth et al., 2019), and the occurrence of sex ratio differences between broods/generations and between nests is deemed too rare to have had any effect on the evolution of eusociality in the Hymenoptera (Gardner et al., 2012).

A simpler explanation for the strong association between a loss of prepupal diapause and the evolution of eusociality reported here is that eusociality evolves via the subsocial route and subsociality is defined as females interacting with their adult offspring. Thus, a loss of prepupal diapause is simply a prerequisite for subsociality. Indeed, bee species with prepupal diapause are solitary and not closely related to any eusocial lineages (Danforth et al., 2019). In contrast, as shown in this and other studies (Danforth et al., 2019; Santos et al., 2019), all subsocial bees exhibit adult diapause or no diapause. Similarly, wasps with prepupal diapause appear to be exclusively solitary (Fye, 1965; Brockmann and Grafen, 1992), whereas subsocial species exhibit adult or no diapause.

Life cycles in which daughters may either enter prepupal diapause or complete development and breed in the same season appear to be bet-hedging strategies in unpredictable environments (Fye, 1965; Brockmann and Grafen, 1992; Danforth et al., 2019). Prepupal diapause is especially common in solitary bees living in arid environments, likely because prepupae are resistant to desiccation, permitting them to remain in diapause for a considerable amount of time, sometimes for several years (Danforth et al., 2019). In more predictable and less arid environments, females may be selected to complete development with the option of entering diapause as adults. One advantage of adult diapause is that it permits females to begin nesting earlier in the next season than females that had entered diapause as prepupae and must first complete development (Matthews, 1991; Brockmann, 1997; O'Neill, 2001;

Danforth et al., 2019). Earlier emergence from adult diapause in the solitary mining bee *Andrena vaga* is associated with a longer lifespan (Straka et al., 2014). This scenario may favor eusociality if it permits the production of two broods, thus allowing first-brood females the opportunity to raise second-brood reproductive siblings. There are several species of bees that are primitively eusocial at low latitudes or elevations, where the length of the breeding season permits the production of two broods, but are otherwise subsocial (Purcell, 2011).

In addition, with adult diapause, if a first-brood daughter has no opportunity to breed either in her natal nest or independently, because of a high cost of independent breeding, and thus her only options are to enter diapause or to help raise the second brood, the selective barrier to helping is extremely low (**Figure 8**). This univoltine lifecycle is observed in paper wasps (Polistinae) and sweat bees (Halictinae) (Yanega, 1988; Reeve et al., 1998; Schwarz et al., 2007). In this situation, a first-brood daughter is in a genetic sense simply trading off herself against her contribution to the current production of full siblings in the second brood; she would have to help produce only two full siblings to come out even with an even sex ratio because each sibling is related to her by one half on average. In other words, the cost of helping in terms of personal reproduction is simply the female's genome; her future reproduction, had she opted to enter diapause, is not relevant since any siblings she helps to produce may also breed in the future. This conclusion was derived by Pamilo (1991) using a formal inclusive fitness model, but has apparently not attracted any attention. The idea is supported by the observation that in vespid wasps and bumblebees the ontogeny of gynes, but not workers, is guided toward an adult diapause phenotype (Hunt and Amdam, 2005; Hunt et al., 2007; Amsalem et al., 2015; Piekarski et al., 2018). The idea is also supported by what may represent a step on the path to full eusociality: brood divalency in the sweat bee *Halictus rubicundus*, in which some first-brood females become workers while others mate and enter diapause (Yanega, 1988). The hypothesis that adult diapause favors eusociality in this manner could potentially be tested in the Halictinae, which exhibit considerable inter- and intra-specific variation in life cycles and social systems that is often associated with season length (Yanega, 1997; Schwarz et al., 2007; Field et al., 2010; Purcell, 2011; Kocher et al., 2018).

Obligate Eusociality Is a Major Evolutionary Transition

A major evolutionary transition is a change in the way genetic information is transmitted between generations (Maynard Smith and Szathmáry, 1995). Such transitions help explain the hierarchical nature of living systems, including the origins of new levels of individuality, such as unicells, eukaryotic cells, multicellular organisms, and eusocial societies (Bourke, 2011; West et al., 2015). Therefore, to qualify as a major evolutionary transition, eusociality must be obligate, in the sense that reproduction cannot occur outside the social group, thus defining the social group as an individual or superorganism. Boomsma and Gawne (2018) suggest defining such groups by workers remaining unmated throughout their lives, which is associated with distinct morphological differences between queens and workers. This allows for worker production of males. I believe this is insufficient, however, since it leaves open the possibility that a foundress may reproduce independently of workers. Crespi and Yanega (1995) define obligate eusociality as a lack of totipotency of both the reproductive and non-reproductive castes, making castes mutually dependent, thus creating a new level of individuality. Crespi and Yanega (1995) define castes by the commitment of individuals throughout adulthood to a behavioral role, usually involving a distinct morphology, in order to distinguish eusociality from cooperative breeding, in which individuals, usually offspring, act as helpers for only their early adult years. The requirement for commitment to a caste by Crespi and Yanega (1995) and Boomsma and Gawne (2018) is motivated by the analogy with multicellular organisms, with their distinct germ line and soma. However, then this requirement seems too stringent since in both animals and plants reproduction sometimes occurs through somatic tissue. For example, cnidarians such as *Hydra*, may reproduce by budding, and plants commonly reproduce vegetatively.

If we disregard commitment to caste, then the swarm-founding Polistinae (paper wasps) (Jeanne, 1991), which rely on workers to establish new nests, but in which workers may become queens (Strassmann et al., 2002), are nevertheless obligately eusocial and thus would meet the criteria for a new level of individuality. On this basis, in addition to the swarm-founding Polistinae, the non-parasitic bumblebees, stingless bees, honeybees, non-parasitic hornets, and yellowjackets and most ants are obligately eusocial (Crespi and Yanega, 1995; Boomsma and Gawne, 2018). Thus, in the Hymenoptera there have been five cases of a major evolutionary transition to obligate eusociality, in the MRCAs of the clades Bombini + Meliponini, Apini, Formicidae, Vespinae, and Epiponini (**Figure 9**).

The process of a major evolutionary transition to a new level of individuality can be broken down into three steps: social group formation, social group maintenance, and social group transformation (Bourke, 2011). The first step, social group formation, has been the focus of the present attempt to understand the life history factors that favor the origin of eusociality, in the form of primitive eusociality. The third step, social group transformation, is the set of processes that transforms a stable social group (primitive eusociality) to an obligate social group, and is the stage of a major evolutionary transition where the new level of individuality emerges. In the case of obligate eusociality, none of the life history characters studied here are associated directly with social group transformation. However, unlike facultatively eusocial clades, and with the exception of bumblebees, obligately eusocial clades are characterized by species that form very large colonies. This supports the size-complexity hypothesis (Bourke, 2011). In a larger group, an individual worker has a lower probability of attaining reproductive status, thus selecting for greater reproductive altruism, and because of relatedness asymmetries, for worker policing of worker reproduction. Such specialization permits the production of larger groups, which selects for even greater reproductive division of labor, producing a positive feedback loop. The size-complexity hypothesis is supported in the

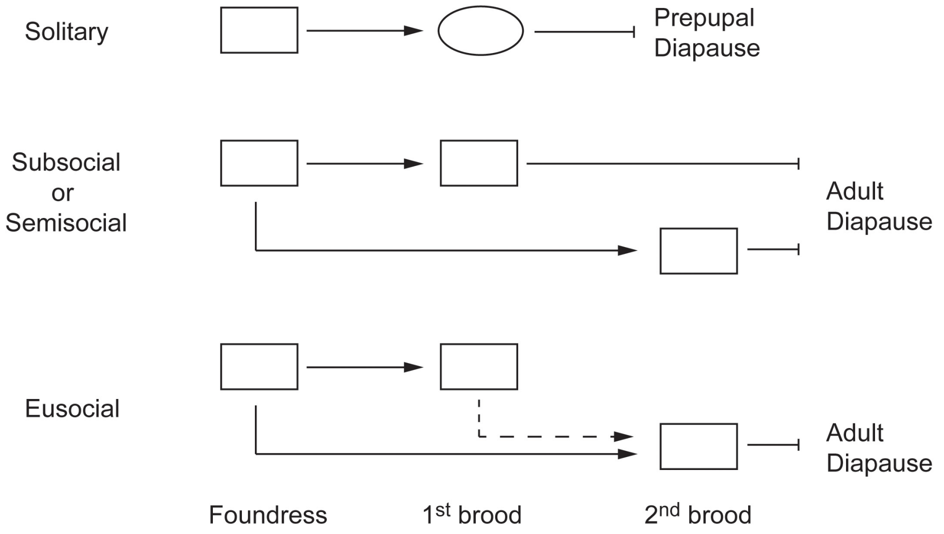


FIGURE 8 | Schematic diagrams of life cycles with prepupal and adult diapause. Adults are represented by rectangles and prepupae are represented by an oval. Solid arrows indicate breeding, and the dashed arrow indicates helping. The distinction between subsociality and semisociality is whether there is a single foundress (subsocial) or multiple foundresses (semisocial).

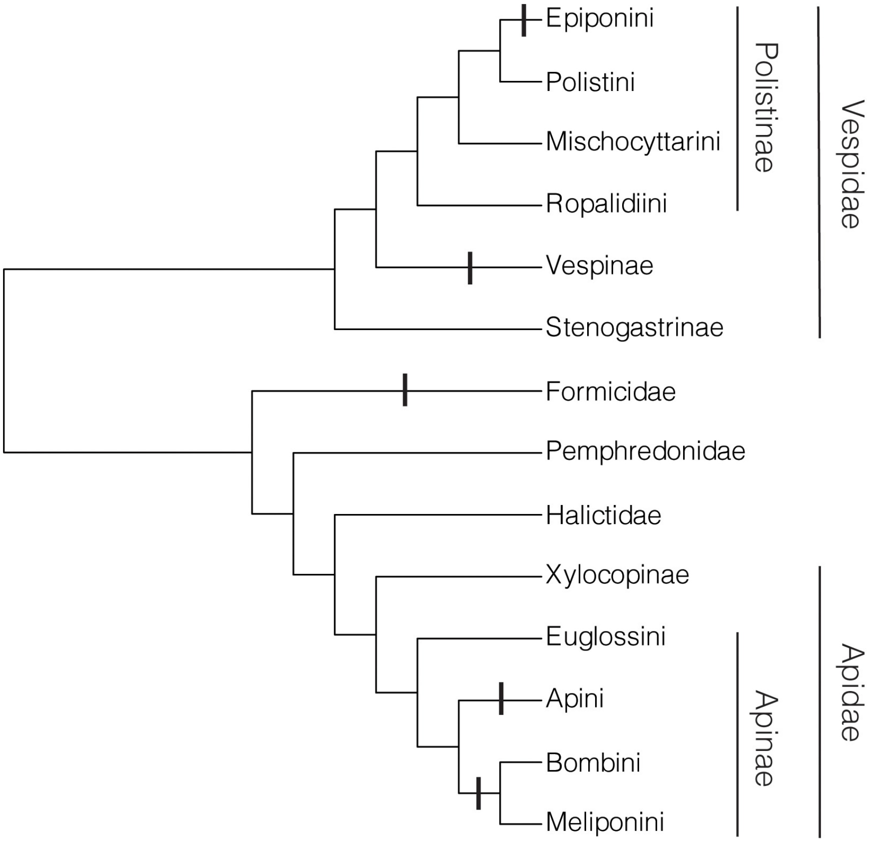


FIGURE 9 | Major evolutionary transitions to obligate eusociality. Vertical bars indicate transitions. Cladogram of groups exhibiting eusociality is based on recent phylogenies (Peters et al., 2017; Piekarski et al., 2018; Sann et al., 2018; Bossert et al., 2019).

Hymenoptera by positive correlations with colony size for social complexity and morphological and lifespan differences between castes (Fjerdingstad and Crozier, 2006; Rodriguez-Serrano et al., 2012; Ferguson-Gow et al., 2014; da Silva, in press).

An interesting consequence of the major evolutionary transition to obligate eusociality is that inclusive fitness accounting (Hamilton, 1964a) can no longer be used to explain the evolutionary forces maintaining the social group. Inclusive fitness is calculated as the reproductive output of a focal individual (or mated pair), exclusive of any offspring produced due to help from others, plus any additional offspring produced by others due to the focal individual's help, weighted by the relatedness between the focal individual and those receiving help. This means that a queen in an obligately eusocial species has no inclusive fitness, since all her reproduction is dependent on help from workers (Queller, 1996a). This does not imply that kin selection no longer operates; it simply reflects that inclusive fitness, which is calculated for individuals, must now be calculate for the new level of individual, the colony. I suggest that the inclusive fitness of an obligately eusocial colony may help explain the paradoxical evolution of super-colonies of ants (Helanterä et al., 2009).

CONCLUSION

This study represents the most comprehensive assessment of the transitions to eusociality in the Hymenoptera to date. Eusociality has evolved more frequently in the Apidae and Halictidae than previously thought. Interestingly, semisociality either precedes or possibly precedes every transition to eusociality outside the Vespidae. Given that semisociality should be favored by haplodiploidy, this pattern may help explain why eusociality is common in the Hymenoptera. Although ancestral state reconstruction and statistical tests of transition rate dependencies show that different life histories may favor eusociality in different families, a loss of prepupal diapause appears to be a general prerequisite. The association with a loss of prepupal diapause may simply reflect the subsocial route to eusociality, both of which may be associated with an extension of the breeding season that permits the production of more than one brood. An intriguing possibility is that with adult diapause a first-brood female that does not have the option of breeding immediately has a very low selective barrier to becoming a worker because she is simply trading off herself against any second-brood offspring she helps to raise. A fuller understanding of the conditions favoring eusociality will require knowledge of the interactions between life cycles and season length that favor first-brood females that opt to help rather than breed or enter diapause. Obligate eusociality, which meets the definition of a major evolutionary transition, has evolved five times in the Hymenoptera. And consistent with the evolution of a new level of individuality, inclusive fitness can no longer be calculated for individuals in obligately eusocial groups but must be calculated at the new level of individuality, the colony.

DATA AVAILABILITY STATEMENT

Data are available in figshare: https://doi.org/10.6084/m9.figshare.16691290.v1.

AUTHOR CONTRIBUTIONS

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.727124/full#supplementary-material

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Identifying Causes of Social Evolution: Contextual Analysis, the Price Approach, and Multilevel Selection

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Kin selection theory and multilevel selection theory are distinct approaches to explaining the evolution of social traits. The latter claims that it is useful to regard selection as a process that can occur on multiple levels of organisation such as the level of individuals and the level of groups. This is reflected in a decomposition of fitness into an individual component and a group component. This multilevel view is central to understanding and characterising evolutionary transitions in individuality, e.g., from unicellular life to multicellular organisms, but currently suffers from the lack of a consistent, quantifiable measure. Specifically, the two major statistical tools to determine the coefficients of such a decomposition, the multilevel Price equation and contextual analysis, are inconsistent and may disagree on whether group selection is present. Here we show that the reason for the discrepancies is that underlying the multilevel Price equation and contextual analysis are two non-equivalent causal models for the generation of individual fitness effects (thus leaving different "remainders" explained by group effects). While the multilevel Price equation assumes that the individual effect of a trait determines an individual's relative success within a group, contextual analysis posits that the individual effect is context-independent. Since these different assumptions reflect claims about the causal structure of the system, the correct approach cannot be determined on general theoretical or statistical grounds but must be identified by experimental intervention. We outline interventions that reveal the underlying causal structure and thus facilitate choosing the appropriate approach. We note that kin selection theory with its focus on the individual is immune to such inconsistency because it does not address causal structure with respect to levels of organisation. In contrast, our analysis of the two approaches to measuring group selection demonstrates that multilevel selection theory adds meaningful (falsifiable) causal structure to explain the sources of individual fitness and thereby constitutes a proper refinement of kin selection theory. Taking such refined causal structure into account seems indispensable for studying evolutionary transitions in individuality because these transitions are characterised by changes in the selection pressures that act on the respective levels.

Keywords: social evolution, multilevel selection, causal model, Price equation, kin selection

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1. INTRODUCTION

When individual traits have effects on other individuals, individual fitness depends not only on self but also on the social environment, i.e., interaction partners. Kin selection theory (KS) deals with this problem by regarding the social environment as an external factor that, together with direct fitness effects of a trait, determines evolutionary dynamics with respect to selection. By assuming a certain correlation between trait value of an individual and average trait value of its social environment, e.g., through relatedness, Hamilton's rule can be formulated and answers the question of whether a trait with direct and indirect effects increases or decreases in frequency given the organisation of the population, i.e., the parameter of relatedness *r* (Frank, 1997). In short, KS acknowledges indirect effects (for which it was developed) but focuses on how relatedness affects individual fitness and is indifferent to the levels on which selection acts.

Multilevel selection theory (MLS) differs from this picture in that it posits the social environment as a unit, e.g., the group, that can be subject to selection acting at a level above that of individuals (Wilson, 1975; Wade, 1976, 1978; Uyenoyama and Feldman, 1980; Wilson and Sober, 1989). The theory thus promotes the concept of a group from a mere collection of individuals targeted by similar selection pressures to a unit that has a causal role in the selection process. More precisely, MLS theory understands a group as a unit whose interaction with the selective environment—through properties of the group as a whole—causally affects the fitness of its individual subunits (Wade and Kalisz, 1990). This means that individual fitness is a composite quantity determined by two factors: the individual effect of the trait and an effect on the group that an individual is a part of, and via this group effect, on the individual itself. The MLS view is not in opposition with KS but merely highlights that selection at the group level may be part of a causal mechanism resulting in individual fitness differences and must be taken into account if we want to understand the source of individual fitness differences (Dugatkin and Reeve, 1994; Sober and Wilson, 1994). Put differently, while KS is content with determining inclusive fitness at the individual level, MLS claims that individual traits can have effects that are best understood as group effects. Note that KS and MLS make the same predictions about trait-frequency dynamics on the individual level because selection at higher levels entails selection at lower levels and KS interprets all selection in individual terms. The explanatory goals of KS itself (Okasha, 2015; Marshall, 2016) derive largely from the goal of establishing inclusive fitness as a quantity maximised by evolution (Hamilton, 1964). Here, we refer to KS as a model that is free of assumptions regarding the level of selection in the sense that KS subsumes all selection at the individual level while MLS deviates from this model by assigning selection to several levels. While MLS aims to analyse the proximate causal structure of selection at multiple levels of organisation, KS establishes the direction of trait-frequency change based on individual fitness consequences of the trait and the relatedness structure of the population.

The distinction between individual effects and group effects of individual traits presents MLS with a problem not encountered by KS: how can the presence of a group effect be detected empirically/statistically and how can the strength of the group effect be quantified in comparison to the individual effect of the trait. After all, the claim that group effects determine individual fitness can only be of use if such effects can be detected empirically. To give an example, Eldakar et al. (2010) claim that the fitness of male water striders *Aquarius remigis* organised into patches (also referred to as social environments or groups) depends on two components that are both affected by an aggressiveness trait individually expressed by the males. The individual component is given by the positive effect of aggressiveness on fitness mediated by mating success which is higher for more aggressive males that secure more mating opportunities than less aggressive males (Watters and Sih, 2005). The group component of individual fitness, on the other hand, arises from a different causal pathway and represents a negative effect of aggressiveness on fitness. Since the harassment experienced by females on a patch reflects the cumulative male aggression level on that patch and females tend to avoid harassment by escaping their current patch, the trait has a negative effect on patch productivity by decreasing the number of females on the patch and therefore the reproductive resources of all males on that patch. If such a decomposition into causes of individual fitness is to be useful, this decomposition must be empirically accessible in the sense that fitness is quantitatively given as a function of an individual component and a group component. This is possible only with a valid method of measuring the decomposition in empirical data.

Two methods for carrying out a quantitative decomposition of individual fitness into an individual component and a group component have received particular attention in the literature (Heisler and Damuth, 1987; Goodnight et al., 1992; Frank, 1998; Okasha, 2006; Sober, 2011; McLoone, 2015): the multilevel Price equation and contextual analysis which, following Okasha, we refer to as the "Price approach" and the "contextual approach," respectively. However, the partitions of individual fitness given by the two methods are different in general. In particular, there are cases in which the multilevel Price equation claims the absence of group effects while contextual analysis claims their presence and vice versa.

The inconsistency between the two approaches is problematic because proponents of MLS argue that the distinction between individual effects and group effects is not just a statistical exercise but reflects a separation of causal pathways in the biological system under study as described above. While one causal pathway emanating from the individual trait is proposed to affect only individual aspects of fitness (the fitnesses of the bearer and its interaction partners), a different pathway is claimed to relate the trait with properties of the group as a whole and hence with a group component of individual fitness. Since the desired decomposition must reflect the underlying biological reality, two methods of decomposition that yield different answers cannot both be correct (Sober, 2011). Previous attempts at resolving these discrepancies have been inconclusive, leaving theorists and empiricists applying multilevel selection theory in the unfortunate situation that, even among proponents of multilevel selection theory, there is no unanimously agreed upon method for measuring the strength of group selection in the simplest additive cases (Eldakar et al., 2010; Clarke, 2016). Given that multilevel selection is sometimes viewed as contentious in traditional evolutionary theory, and even the proponents of MLS have been unable to agree on a measure (and thus even unable to agree whether a group effect is present or not in a particular case), this may suggest that MLS is not well-understood and should be abandoned in favour of kin selection theory.

The aim of this paper is to show that the essential difference between the Price approach and contextual analysis lies in the causal structure each method posits as underlying the observed measurements of individual fitness. Briefly, while contextual analysis assumes that the individual component is determined by direct fitness effects of the trait only, the Price approach sees the individual component as a result of within-group competition and duly assumes it to be affected by the trait values of group mates. Put differently, contextual analysis assumes that the individual effect of the trait is absolute in the sense that it is independent of the social environment. The Price approach, on the other hand, assumes that the trait affects the competitiveness of its bearer so that its fitness effect is relative in the sense that it depends on the social environment. This difference leads to different remainders to be explained by group effects and thereby to different measurements of the strength of group selection. Recognising that the difference between the two approaches arises from a difference in the underlying model of reality enables us to see how to determine which of the two approaches is correct in a given case, i.e., the one whose underlying model reflects the causal structure of the system that is being studied. In particular, the applicability of the two approaches depends on the biological scenario at hand and cannot be made on theoretical grounds.

Our analysis demonstrates how the application of MLS to a biological scenario requires and formalises an understanding of the system that is not implied by KS. More precisely, MLS introduces a layer in the causal structure that cannot be deduced from the reduced theory. For evolutionary transitions in individuality, understanding this additional causal structure is crucial because how it changes over evolutionary time reflects the transition from independent individuals to integrated groups as selection shifts from the individual to the group level (Godfrey-Smith, 2009; Clarke, 2016).

This paper is organised as follows. First, we show that both contextual analysis and the Price approach can be interpreted in terms of causal graphs that describe how each of the approaches models the dependence of individual and group component of fitness on individual and group trait. We then compare the two approaches using these causal graphs. This allows us to illustrate very clearly why the two approaches give different answers with respect to the strength of group selection. In addition to identifying the source of the discrepancy, our analysis identifies an experimental intervention that reveals which, if any, of the two approaches is correct and shows that neither is always correct. Indeed, the correct approach depends on causal mechanisms in the biological system that cannot be determined based on the distribution of individual fitness over individual trait and group trait without experimental intervention.

2. MODEL

2.1. Fitness, Selection, and the Price Equation

The evolutionary model in which we frame our arguments is as simple as possible whilst being able to support the features we set out to discuss. Individuals are defined by their allele at a biallelic locus, with the two alleles representing the presence and absence of a trait, which also defines their phenotype (denoted by x) and replicate asexually without mutation. A population of individuals is partitioned into non-overlapping groups of equal size such that an individual interacts equally with all members of its group (the assumptions on group size and disjointness are made for convenience only). The absolute fitness of an individual determines per capita growth rate and is a function of its own trait as well as the group trait, but not a function of other properties of the population (absence of, e.g., global frequency dependence; moreover we do not assume a mechanism of global population regulation). The group phenotype is defined as the average phenotype of the individuals within the group. The following considerations concern on bout of selection so that individuals reproduce within their given social environment. We therefore take the groups for granted and do not consider the mechanism of their formation. Taking a causalist stance, we assume that the fitness function is deterministic rather than a statistical abstraction from data (Otsuka, 2016) and stable in its functional form (i.e., the selective environment that determines fitness in interaction with the phenotype is not changing, Wade and Kalisz, 1990). Moreover, we assume that the fitness function is additive such that

$$w = c_1 x + c_2 X, \tag{1}$$

where w denotes the fitness of an individual with phenotype x and group phenotype X, and $c_1, c_2 \in \mathbb{R}$ denote the coefficients of the functional representation of fitness in this simple hypothetical example (fitness and individual phenotype are centered at the population mean). This notation corresponds to the method of direct fitness or neighbour-modulated fitness in KS (Taylor et al., 2007). $c_1 x$ represents the direct fitness effect of the trait on its bearer, $c_2 X$ the indirect fitness effect on trait bearers' interaction partners. However, in contrast to approaches using inclusive fitness, we emphasise a causal viewpoint in this paper by regarding equations such as Equation (1) as structural equations that mirror causal assumptions about the system rather than as regression equations. The assumption of additivity in Equation (1) is a gross simplification that has been criticised because it ignores synergistic effects and therefore only applies to rare cases (Allen et al., 2013; Van Cleve and Akçay, 2014). The aim of this paper, however, is not biological generality but to demonstrate causal distinctions made by contextual analysis and the Price approach. The additive fitness function (1) suffices to show that the two approaches to multilevel selection discussed in this paper yield causally non-equivalent structural equations in this case.

The upper row of diagrams in **Figure 1** shows the crucial difference between non-social and social evolution in terms

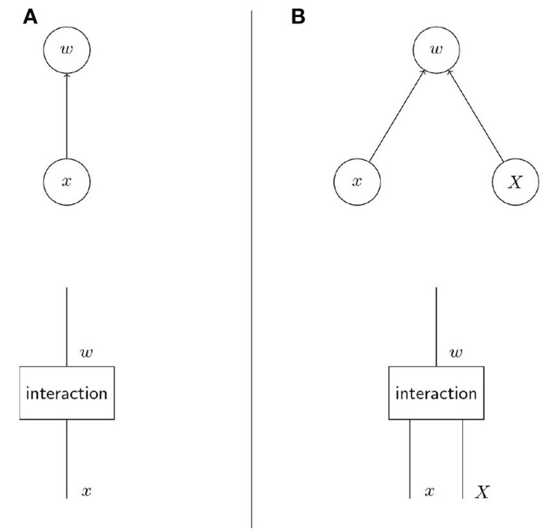


FIGURE 1 | The crucial difference between non-social and social evolution lies in the factors that determine fitness. The diagrams in the upper row are causal graphs that show the dependence of individual fitness on phenotypic factors. The lower row are informal string diagrams (Jacobs et al., 2019) that explicitly represent the process that yields a variable given another variable. In non-social evolution **(A)** individual fitness depends on the individual phenotype only while in social evolution **(B)** individual fitness depends on the social partners' phenotype in addition to the focal individual's phenotype (Taylor et al., 2007). In both cases individual fitness arises from the interaction of the phenotype(s) with the selective environment.

of causal graphs. While individual fitness w depends only on the individual phenotype x in non-social evolution, social evolution introduces a dependency on social partners' phenotype X (Wolf et al., 1999). In Pearl's causal modelling framework, causal graphs as in the upper row of **Figure 1** represent the graphical counterpart of structural equations (Pearl, 2012). These structural equations model the causal process that yields the output variables, here w, given the input variables, here x and X. In contrast to multiple regression these structural equation reflect assumptions about the causal structure of the system. To make the causal process explicit we make informal use of string diagrams (Jacobs et al., 2019) to represent the causal graphs (i.e., Bayesian networks) in the lower row of diagrams in **Figure 1**. These diagrams are read from bottom to top and depict the variables as strings and the structural equations that transform the variables (i.e., the modelled processes) as boxes. The process of interaction of the phenotype with the selective environment is represented as box interaction that determines individual fitness based on individual and group phenotype. The diagrams in **Figure 1** are graphical and non-parametric versions of the structural Equation (1). Both the diagrams and the structural equation describe general features of the causal process proposed to determine the fitness of individuals with phenotype x within a group with phenotype X.

The process of selection in a population is given by the change in trait frequency according to the Price equation without mutation

$$\overline{w}\Delta\overline{x} = \mathrm{Cov}(w, x). \tag{2}$$

Note that we do not assume that groups themselves replicate or can be assigned group fitness over and above the fitness of the individuals that constitute a group. Our model is therefore of MLS1 type in the sense of Heisler and Damuth (1987), i.e., the focus of the analysis is on individuals, group trait and group fitness are averages of the corresponding quantities of the individuals within the group.

The starting point for the analysis of selection in a population in terms of MLS is the observation that an aspect of selection acts on groups as a whole. This means that individual selection is in part determined by the group trait X because selection favours groups with high (or low) group trait. In particular, this aspect of selection is the same for all members of a group and is captured by the process by which some groups contribute more offspring to the next generation than others due to differential proliferation and extinction (Uyenoyama and Feldman, 1980; Wade and Goodnight, 1998). Note that it makes no difference to the change in trait frequency whether (an aspect of) selection acts on the group as a whole or on all group members individually but in the same way. However, the aim of MLS is not only the prediction of outcomes but also the attainment of a causal understanding of the selection process (Sober and Wilson, 1994). This understanding comprises selective processes at the individual and the group level: individual fitness not only *depends* on a group trait in addition to the individual trait but fitness also *arises* as consequence of a process that affects the group as a whole in addition to a process that affects each individual specifically. Acknowledging the latter of these dual viewpoints is characteristic of multilevel selection theory as opposed to kin selection theory. The neighbour-modulated approach to kin selection, for instance, formalises the fitness effects of the social environment as factor that alters individual fitness but doesn't view the group as interacting with its own selective environment (Taylor et al., 2007).

Explanations for the evolution of cooperative traits, i.e., individual traits that are costly for their bearers in comparison with non-bearers, often rely on the interplay between two processes of this kind. In microcolonies of the bacterium *Pseudomonas aeruginosa*, for instance, the production of siderophores puts individuals at a fitness disadvantage because the process of producing the metabolite binds resources that could otherwise be used for reproduction (Weigert and Kümmerli, 2017). However, the secreted siderophores are shared within the microcolony and thus increase colony fitness due to their iron-scavenging function. Total fitness of individuals results as the combination of the two processes acting on the individual directly and on its group.

In **Figure 1**, the causal graph for social evolution in **Figure 2** is refined to a causal graph and a corresponding string diagram that explicitly represent the distinct processes of interaction with the selective environments on the individual level and the group level. The box "combine" in the lower diagram in **Figure 2** corresponds to a function that combines the outcomes

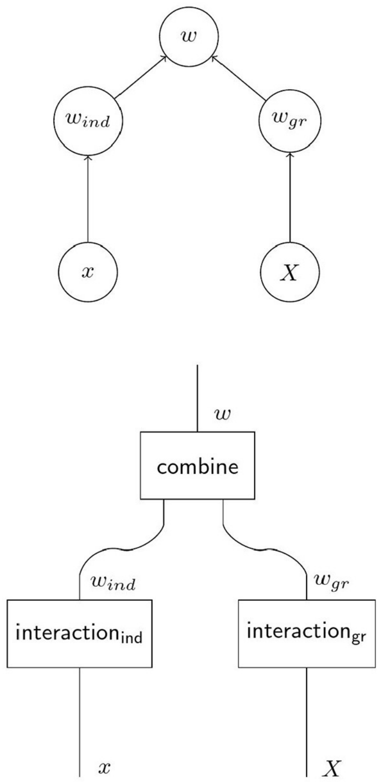


FIGURE 2 | When multilevel selection (MLS1) operates, individual fitness may not only depend on the individual trait and the group trait as in **Figure 1B**. A model of MLS1 usually involves two distinct processes whose combined outcomes yield individual fitness. The individual component of fitness w_{ind} is the outcome of the individual's interaction with its selective environment as in non-social evolution. But also the group as a whole and through its group phenotype X interacts with a selective environment in a causally distinct process that yields the group component of fitness w_{gr}. Total fitness arises as combination of the two processes.

of the two processes into total individual fitness. For a complete specification of the model in terms of structural equations this function must be specified in addition to structural equations for the processes on the individual and the group levels. As we will argue in the next section 2.2, the causal models corresponding to contextual analysis and the Price approach combine their respective structural equations for the individual and group level interaction with the selective environment additively, so that

$$w = w_{\text{ind}} + w_{\text{gr}}. \tag{3}$$

The purpose of the decomposition in Equation (3) is to explicitly and formally acknowledge the basic tenet of MLS that fitness (here at the individual level) is determined not only by how the individual interacts with its selective environment but also by how the individual's group interacts with the selective environment on a level above that of the individual. We introduce w_{gr} to formally capture fitness effects that result from the interaction of the group as a whole with the selective environment. The quantities w_{ind} and w_{gr} are proxies for the effects of causal processes, the former for processes that affect individual fitness specifically for each individual, the latter for processes that affect the group. The decomposition in Equation (3) additive because of the simple fitness function chosen in Equation (1). While an MLS analysis always rests on a decomposition of fitness into contributions from various levels, this decomposition is, generally, not additive. The difference between the two approaches with respect to the arena of individual selection, which is the global population for contextual analysis and the local group for the Price approach, holds more generally, regardless of whether individual and group selection are combined additively.

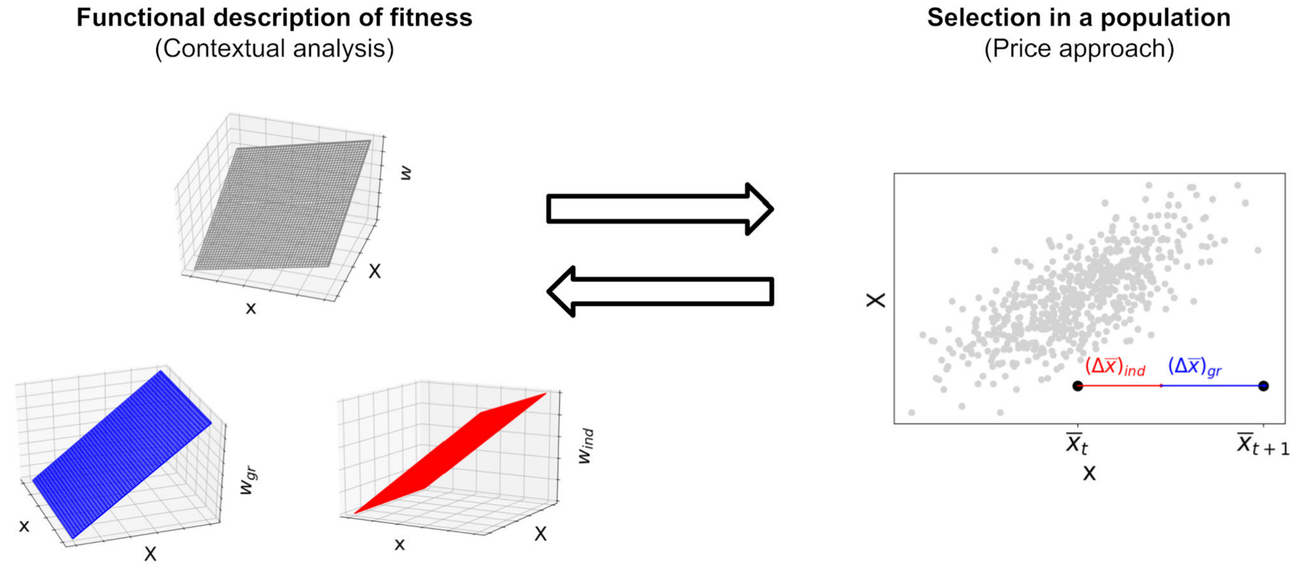
Since the Price equation is linear in the fitness argument, the decomposition expressed in Equation (3) corresponds to a decomposition of the strength of selection itself

$$\overline{w}\Delta\overline{x} = \overline{w}(\Delta\overline{x})_{ind} + \overline{w}(\Delta\overline{x})_{gr} = \mathrm{Cov}(w_{\text{ind}}, x) + \mathrm{Cov}(w_{\text{gr}}, x)$$

In order to make quantitative statements about the strengths of group selection vs. individual selection, an MLS analysis must determine the components in this decomposition. However, while individual trait and fitness as well as aggregates thereof can be measured directly, individual effect and group effect, or their covariance with the individual trait, are generally not amenable to direct measurement. The multilevel Price equation and contextual analysis are two methods of obtaining w_{ind} and w_{gr} by statistical means given individual traits and fitnesses (Okasha, 2006).

2.2. Contextual Analysis and the Price Approach

Equation (1) partitions individual fitness into the effects of the individual trait and the group trait. It describes how two phenotypic traits combine to yield another trait of the individual, namely absolute fitness. Contextual analysis (Heisler and Damuth, 1987; Okasha, 2006; note that here and the following we refer by "contextual analysis" to the standard structural equation with the untransformed variables x and X, as is customary in discussions on the issues reported here) takes effects of the group trait in Equation (1) as indicating group selection. Strictly speaking, $c_2 \neq 0$ in Equation (1) implies the potential of the trait to undergo group selection conditional on the existence of group-trait variation between groups (Wolf et al., 1999, see McLoone, 2015 for a discussion of this difference). We regard group effects on fitness as more fundamental than a concept of group selection itself as the former do not depend on properties of a population but reflect causal processes that increase or decrease reproductive success of an individual situated in a group context vis-à-vis a specific selection regime that in turn determines individual fitness. Group effects can lead to group selection if, in a specific population, they generate fitness differences between individuals. This requires $\mathrm{Var}(X) \neq 0$, for if $\mathrm{Var}(X) = 0$ all individuals have the same group trait and are therefore subject to the same group effects. Given a population of individuals and a fitness function that yields individual fitness as superposition of fitness effects of the variables that causally determine fitness, the Price equation yields



Functional description of fitness (Contextual analysis)

Selection in a population (Price approach)

FIGURE 3 | In the **left** panel, contextual analysis decomposes the fitness function into an individual and a group component that depend on the individual trait and the group trait, resp. In the **right** panel, the multilevel Price equation decomposes selection, that is a change in mean phenotype in the population, into a change due to individual selection and a change due to group selection. The standard Price equation without transmission bias maps a functional description of fitness to a process of selection in a population of individuals whose fitness is defined by this functional description. Since the Price equation is given by a covariance and therefore linear in the fitness function, a decomposition of fitness into the summands individual and group effects yields a corresponding and unique decomposition of selection in a population into the summands individual and group selection, each contributing a change in mean phenotype. In section 2.2, we show that, conversely, the coefficients of a decomposition of selection into individual and group selection using the Price approach correspond to coefficients of a structural equation for fitness, given the assumption that fitness is linear as in Equation (1).

the effect of selection on this population, that is the change in mean phenotype over one generation (**Figure 3**).

The Price approach to multilevel selection (Price, 1972; Okasha, 2006; Gardner, 2015) rests on the partition of selection itself given by the multilevel expansion of the Price Equation (2)

$$\overline{w}\Delta\overline{x} = \mathrm{Cov}(W, X) + \mathrm{E}[\mathrm{Cov}_{\mathrm{wg}}(w, x)] \tag{4}$$

and posits that a population is undergoing group selection if the first term in Equation (4) is non-zero. In light of our remarks regarding group effects and group selection above, the Price approach and contextual analysis therefore decompose different quantities and are not directly comparable. However, this difference is superficial as the partition of fitness effects given by contextual analysis corresponds to a partition of selection and the partition of selection given by the multilevel Price equation corresponds to a partition of fitness effects. Contextual analysis, i.e., the functional representation of fitness in Equation (1), determines selection according to Equation (2) for a population that is partitioned into groups: given a population of individuals $i \in 1, \ldots, n$ with fitnesses

$$w_i = c_1 x_i + c_2 X_i,$$

where X_i is the trait of the group the i^{th} individual is part of, the change in mean trait value in the population follows from Equation (2) as (Okasha, 2004),

$$\overline{w}\Delta\overline{x} = c_1 \mathrm{Var}(x) + c_2 \mathrm{Var}(X). \tag{5}$$

Thus the decomposition of fitness into individual and group effects given by contextual analysis corresponds to a decomposition of selection whose components, according to contextual analysis, represent the component of individual selection $c_1\mathrm{Var}(x)$ and the component of group selection $c_2\mathrm{Var}(X)$. Conversely, the components of individual selection and group selection according to the Price approach for a population with non-vanishing variance within and between groups correspond to a decomposition of individual fitness into a component of individual effects and group effects. To see how, note that with $w = c_1 x + c_2 X$ (Equation 1),

$$\mathrm{Cov}(W, X) = (c_1 + c_2)\mathrm{Var}(X) \tag{6}$$

(Okasha, 2006; p. 89). Using Equations (5) and (6), the decomposition according to Equation (4) is

$$\begin{aligned}\overline{w}\Delta\overline{x} &= \mathrm{Cov}(W, X)\\ &\quad + \mathrm{E}[\mathrm{Cov}_{\mathrm{wg}}(w, x)] = (c_1 + c_2)\mathrm{Var}(X) + \mathrm{E}[\mathrm{Cov}_{\mathrm{wg}}(w, x)]\\ &= c_1\mathrm{Var}(x) + c_2\mathrm{Var}(X)\end{aligned}$$

and therefore

$$\mathrm{E}[\mathrm{Cov}_{\mathrm{wg}}(w, x)] = c_1(\mathrm{Var}(x) - \mathrm{Var}(X)).$$

Hence the decomposition of fitness

$$w = c_1'(x - X) + c_2'X \tag{7}$$

corresponds to the decomposition of selection

$$\mathrm{Cov}(w, x) = \mathrm{Cov}(c_1'(x - X) + c_2'X, x) = c_1'(\mathrm{Var}(x) - \mathrm{Var}(X))$$

$$+c_2'\mathrm{Var}(X),$$

where $c_1' = c_1$ and $c_2' = c_1 + c_2$.

Through this correspondence both contextual analysis and the Price approach yield decompositions of fitness effects as well as of selection (see **Table 1**). Note that the possibility of conducting contextual analysis with respect to the variables x—X and X rather than x and X—the former choice of variables being equivalent to the Price approach, the latter to contextual analysis—is discussed in Heisler and Damuth (1987) along with examples of circumstances under which this might be causally adequate.

It should be noted that while the interpretation of contextual analysis as structural equation based on causal assumptions is natural, the Price equation and its multilevel expansion are usually viewed as mere statistical identities. However, a causal interpretation of the coefficients of the multilevel Price equation as indicators of selection on the individual and group level as in (Okasha, 2006) requires the assumption of a causal process that gives rise to the measured coefficients. As demonstrated in this section above for the additive fitness function given by Equation (1) the structural equation for a process corresponding to the multilevel Price equation is given by Equation (7).

2.3. Causal Intuitions Underlying an MLS Analysis

A core idea of social evolution is that an individual trait of social organisms has fitness effects not only on its bearer but also on the social environment of the bearer. Common to paradigmatic examples of group selection is an individual trait with effects that change individual fitness homogeneously across the group such that these effects are best viewed as group effects (Sober, 1980). For the water striders described in Eldakar et al. (2010) the exodus of females from patches with high levels of aggressiveness is a group effect of the trait "aggressiveness" in males. This group effect is negative because group productivity is assumed to decrease with the number of females on a patch as females provide reproductive resources. On the other hand, aggressiveness has a positive individual effect because aggressive males secure more mating opportunities. Whether contextual analysis or the Price approach is appropriate depends on details of this latter mechanism. If, for instance, less aggressive males generally have lower reproduction rates due to female behaviour and independent of other males on the local patch the contextual analysis model may be more appropriate. If, on the other hand, male reproduction is subject to competition within patches where, for example, successful reproduction depends on the ability of males to guard their mates the model suggested by the Price approach may correspond more closely to the actual process. In either case, the causal interpretation of the trait refers to proximate fitness effects of the trait and involves the individual as well as the group it is in but not other groups or the population as a whole. Therefore the causal interpretation takes place on the fitness side rather than on the selection side in **Figure 3**.

Since we assume that fitness is an effect of the individual/group trait an individual exhibits, we can read the equations in the left column of **Table 1** as structural equations that determine fitness given the traits. By the assumption on the additivity of interactions these equations are linear. The interpretation of structural equations is aided by the use of causal graphs, more precisely, directed acyclic graphs with causal rather than correlational interpretation (Pearl, 2009). **Figure 4** shows the causal graphs corresponding to the structural equations in **Table 1**. Since the components w_{ind} and w_{gr} reflect quantities that refer to processes occurring in the biological system studied, the causal graphs constitute models of the underlying reality. For example, the group effect of the aggressiveness trait in water striders is given by the propensity of females to remain on the focal patch and this propensity is a function of mean male aggressiveness in the patch (this function is linear by assumption), i.e., the group trait X. The non-equivalence of the causal graphs (**Figures 4A,B**) reflects a difference in how the individual/group components of individual fitness depend on individual/group trait. It should be noted that the factors x and X are not strictly independent as suggested by omitted arrows between x and X in **Figure 4**. Since the group phenotype is generated collectively by all individuals within a group, x does affect X. The arrows are omitted in **Figure 4** because our arguments focus on that part of the causal structure that determines fitness. Details of how the interaction of individual phenotypes gives rise to the group phenotype are not relevant for the present discussion.

The model of fitness underlying contextual analysis (**Figure 4A**) is based on the assumption that the individual component and the group component of fitness are determined only by the individual trait and the group trait, respectively. This means that fitness differences within groups, i.e., differences in the individual component, are due to the individual trait and independent of the group trait. In that sense contextual analysis assumes the individual effects of the trait to be absolute, i.e., independent of group context. In contrast, the Price approach assumes that the group trait also affects the individual component of fitness in a specific way (see the path coefficients in **Figure 4**). This effect of the group trait on the individual component is equivalent to the assumption that fitness differences within groups are due to competition between group members in which the individual trait determines competitiveness of an individual. Indeed, the functional representation of fitness according to the Price approach from **Table 1**

$$w = c_1'(x - X) + c_2'X$$

shows that the individual component sums to zero over each group and that individuals with higher-than-average trait have a positive individual component (negative if $c_1' < 0$).

TABLE 1 | The decompositions of contextual analysis and the Price approach as individual and group fitness effects $w = w_{\mathrm{ind}} + w_{\mathrm{gr}}$ and as components of selection. The parameters of contextual analysis and the Price approach are linked by the equations $c'_1 = c_1$ and $c'_2 = c_1 + c_2$.

	Fitness	**Selection**
	Individual component + **group component**	**Individual selection** + **group selection**
	$w = w_{ind} + w_{gr}$	$\Delta\bar{x} = (\Delta\bar{x})_{ind} + (\Delta\bar{x})_{gr}$
Contextual analysis	$c_1 x + c_2 X$	$c_1 \mathrm{Var}(x) + c_2 \mathrm{Var}(X)$
Price approach	$c'_1(x - X) + c'_2 X$	$c'_1(\mathrm{Var}(x) - \mathrm{Var}(X)) + c'_2 \mathrm{Var}(X)$

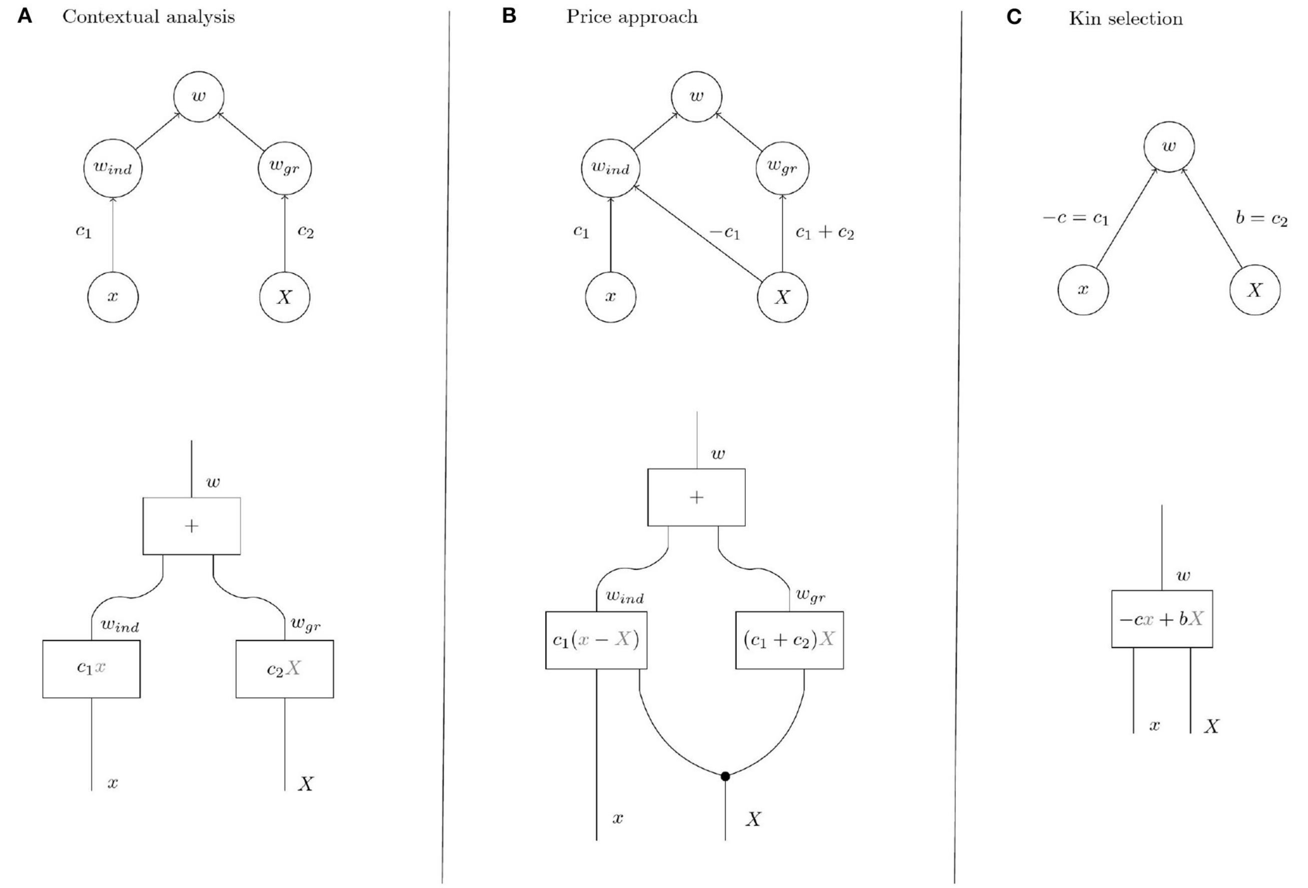


FIGURE 4 | Upper row: Causal graphs showing the interdependence of the variables x (individual trait), X (group trait, i.e., group mean of individual trait), w (individual fitness), w_{ind} (individual component of individual fitness), and w_{gr} (group component of individual fitness). Group fitness W is given as average over the individual fitnesses in a population. **(A)** Contextual analysis assumes an absolute individual effect of the trait. **(B)** In the Price approach, the trait is assumed to have a relative effect in the sense that the trait affects fitness depending on the trait expression of other members of the group. **(C)** In contrast, kin selection theory acknowledges the possibility of indirect effects in addition to direct effects but makes no further assumptions on the causal structure. In KS, it is customary to denote the direct effect of the trait on its bearer by $-c$ and the indirect effect by b. The parameter of relatedness r represents the correlation between x and X and is not pictured in the graph because we focus on selection rather than on properties of group composition. Also the effect of individual phenotype on group phenotype has been omitted, see text. Lower row: String diagrams making the processes that yield the output variables given the input variables explicit. Inside the boxes are the structural equations that mirror the respective processes numerically. In diagrams **(A,B)**, the fitness effect of the group process interaction$_{\mathrm{gr}}$ is proportional to the group phenotype. In diagram **(B)** for the Price approach, the group phenotype X is "copied" at the black dot because it is involved in both processes. In interaction$_{\mathrm{ind}}$ instantiated with the structural equation $c_1(x - X)$, the group phenotype renders the effect of the individual phenotype relative to the group so that individuals, via their individual phenotype, compete within groups.

In other words, the trait affects individual fitness not by generally increasing or decreasing its bearer's fitness but by increasing or decreasing its bearer's competitive ability within the group. We discuss examples of these differences in the next section.

3. RESULTS

3.1. Cases of Disagreement

When comparing the Price approach and contextual analysis it should be kept in mind that both aim to quantify group

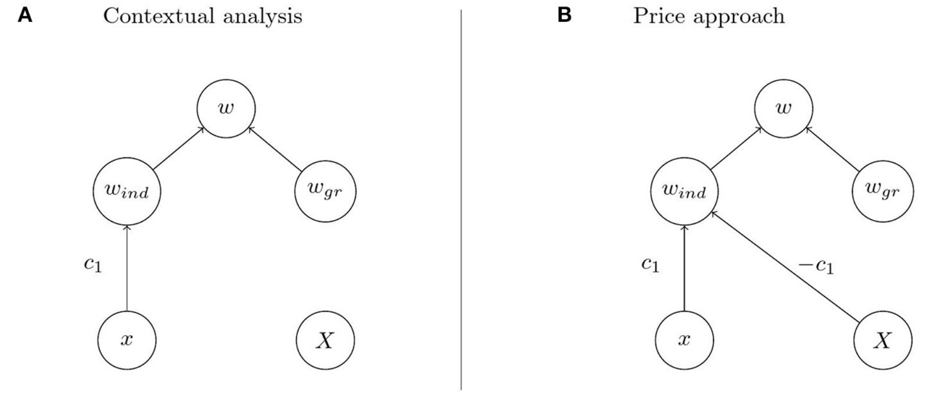


FIGURE 5 | Modified causal graphs when suppressing the effect of the group trait on the group component. **(A,B)** Contextual analysis predicts fitness to be independent of the group trait when the group effect is suppressed while the Price approach does not.

selection and therefore start with the intuitive identification of an effect of the trait on a group-level property that affects fitness of all individuals within a group homogeneously. In the water strider example, an MLS analysis is based on the assertion, intuitively acquired by inspection of the empirical system, that the group mean of the trait "aggressiveness" affects the number of females in a group and therefore the productivity of the group as a whole. This assertion is independent of the subsequent choice of statistical approach to quantifying the strength of group selection. Contextual analysis and the Price approach therefore agree on the nature of the group effect on fitness w_{gr} and on the mechanism bringing forth this effect, though not on its magnitude. The difference between the two approaches lies in the question of which factors affect the individual component of fitness, i.e., which factors are responsible for within-group differences in fitness.

The problem cases for contextual analysis and the Price approach discussed by Okasha (2006) and others (Heisler and Damuth, 1987; Sober, 2011; Goodnight, 2015) reveal issues with the two approaches because the intuition about the level on which fitness effects occur is inconsistent with the verdict of one of the approaches with respect to the strengths of individual and group selection. This intuition is best understood in terms of fitness effects and not in terms of selection because it is based on a mechanism that mediates an effect of the trait on the group component of absolute fitness and is therefore independent of composition and organisation of the population as a whole. Changing a patch of water striders to exhibit a lower level of the group trait "aggressiveness" increases group fitness because less females will flee the patch. This causal explanation of the biological scenario is the core of an MLS analysis and it is independent of other patches and selection dynamics in the overall population. We conclude that the intuition with regard to the levels on which selection acts is about the mechanisms and not about frequency changes in the population. Accordingly, the following discussion is couched in terms of the left-hand side of **Figure 3**.

In the following examples, we determine the coefficients c_1, c_2 of the kin selection model (**Figure 4C**) and discuss their interpretation in terms of the refined models provided by contextual analysis and the Price approach (models in **Figures 4A,B**).

3.1.1. Non-social Trait

A trait is non-social if the fitness of an individual does not depend on the trait values of its interaction partners (group mates) (Okasha, 2006) so that $c_2 = 0$ and $w = c_1 x$ ($c_1 \neq 0$ unless the trait is altogether neutral) in **Figure 4**. Intuitively, a trait of this type cannot be subject to group selection, because it has no fitness effects on its bearer's interaction partners and therefore cannot affect the group component of fitness. However, the causal graph that represents the assumptions of the Price approach (**Figure 4B**) shows an effect of group trait on group component of fitness with weight $c_1 + c_2 = c_1$ and therefore detects group selection where intuitively there is none. Group effects of this type have been called cross-level by-products (Okasha, 2006) and will be discussed in a later section. Note that the causal graph underlying contextual analysis correctly shows the absence of group effects.

3.1.2. Soft Selection

The tension between the Price approach and contextual analysis is reversed in the case of soft selection (Wade, 1985; Goodnight et al., 1992; Débarre and Gandon, 2011). Briefly, soft selection occurs in a group-structured population if mean individual fitness is homogeneous across groups, i.e., if all groups have the same reproductive output. Soft selection models situations in which individuals of each group share a fixed resource and the trait under soft selection determines how an individual fares in the within-group competition for this resource. The group trait determines competitiveness of the group, i.e., mean competitiveness of its members, in the sense that an individual has lower fitness in a competitive group than in a group with low group trait. Soft selection is intuitively considered to be free of group selection (Wade, 1985; Okasha, 2006; Sober, 2011).

The trait has no effect on the group level because changing the trait value of an individual in a group has no homogeneous fitness effect within the group as the change has no consequences for mean group fitness but merely changes the outcome of the within-group competition. It is easy to see that a kin selection model of soft selection takes the form $w = c_1x - c_1X$, i.e., $c_2 = -c_1$, with $c_1 > 0$ (respectively, $c_1 < 0$) if a higher trait value implies higher (respectively, lower) competitiveness. The interpretation of these parameters according to the Price approach yields that the edge from X to w_{gr} has weight $c_1+c_2 = 0$ in the causal graph in **Figure 4B**. The Price approach correctly detects the absence of group selection in this example. However, contextual analysis mistakes the effect of the group trait on fitness as an effect on the group component of fitness according to the causal graph in **Figure 4A**.

Though most researchers that engaged with the problem of inconsistency between contextual analysis and the Price approach seem to agree that no group selection occurs in soft selection, some have argued to the contrary. Goodnight et al. (1992) regard soft selection as an example of group selection since an individual's fitness depends on the trait of the group of which it is a member. We agree that individual fitness depends on the group trait but this effect of the group trait on fitness is an individual effect (the diagonal arrow in **Figure 4B** targets w_{ind}) that represents within-group competition. In soft selection, there is no group effect since the trait does not influence group fitness.

3.1.3. Genotypic Selection With Meiotic Drive

Okasha (2004) introduces "frameshifting" as a desirable property of a general theory of multilevel selection. The theory is capable of frameshifting if it formalises features of group selection in such a way that they hold by analogy whenever the hierarchy given by the group/individual relation is instantiated at other levels of organisation. The treatment of genotypic selection with meiotic drive in MLS terms is relevant in that context because it tests the ability of MLS to frameshift to levels below the level of organisms. Following Wilson (1990) and Okasha (2004) discusses diploid population genetics as an example of multilevel selection where alleles correspond to individuals and diploid genotypes to groups. In this analogy, group effects on allelic fitness are due to genotypic fitness, i.e., organismic fitness of the organism with a specific genotype, and individual effects are due to meiotic drive that creates within-group fitness differences between alleles.

Given the intuition that individual selection as well as group selection is at work in genotypic selection with meiotic drive, the expectation with respect to a decomposition of fitness into individual and group effects is clearly that group selection is present when genotypic fitnesses differ whereas individual selection is brought about by unfair meiosis. However, it is easy to see using specific fitness functions that contextual analysis doesn't agree with intuition in this case. When only meiotic drive is acting while there is no difference in fitness between genotypes, for example, the situation is analogous to soft selection that was shown above to be captured by the Price approach rather than contextual analysis. Furthermore, Okasha (2006) gives the example of two alleles A and B such that genotypic fitnesses are given by $w_{AA} = 4, w_{AB} = 3$, and $w_{BB} = 2$ while meiotic drive causes 2 of the 3 gametes produced by an AB organism to be A. Then fitness of an A allele is 2 and that of a B allele is 1, independent of the genetic background. Thus, despite unfair meiosis and dependence of fitness on the group trait, contextual analysis concludes the absence of group selection, $c_1 \neq 0, c_2 = 0$. The Price approach, in contrast, reaches the correct conclusion that individual fitness is given by $w = \underbrace{c_1(x-X)}_{\text{ind. component}} + \underbrace{c_1X}_{\text{group component}}$ and therefore that both components of selection are non-zero.

Okasha's conclusion that the "covariance approach [i.e., the Price approach] appears to frameshift down quite well, the contextual approach very badly" (Okasha, 2004, p. 498) is thus readily explained by the viewpoint developed so far: unfair meiosis corresponds to the zero-sum game of within-group competition. This is precisely the causal structure assumed by the Price approach.

3.2. Cross-Level By-Products

A core assumption of MLS theory is that a trait an individual expresses may affect properties of its group as a whole and therefore group fitness (i.e., mean individual fitness in a group). This effect is captured by the group component w_{gr} of individual fitness. However, group fitness, in MLS1, is the average individual fitness in a group and therefore comprises not only the group component but also the average individual component w_{ind}. This is problematic because the part of group fitness that entails selection on the group property caused by the trait is w_{gr} only. The contribution of w_{ind} to group fitness is called a cross-level by-product (Okasha, 2006) because it represents fitness of the individuals that constitute the group, i.e., the lower level, rather than fitness that is a property of the group as a whole, i.e., the higher level. Intuitively, a group with many individually fit members seems more fit than a group with few individually fit members even when the group component w_{gr} and therefore the fitness vis-à-vis group selection that is to be quantified is the same for both groups. The non-social trait case discussed above is a good example of this effect. Since there is no group property for group selection to act on in this case, group fitness comprises solely of individual fitness from the level below and therefore consists entirely of cross-level by-products.

To see how contextual analysis and the Price approach handle cross-level by-products assume that individual fitness is given by the expression $w = c_1x + c_2X$. The decomposition of group fitness $W = (c_1 + c_2)X$ into a component due to group effects and a component due to individual effects depends on the causal structure and therefore differs between the two approaches. While contextual analysis partitions group fitness into individual and group component as $W = \underbrace{c_1X}_{\text{ind. component}} + \underbrace{c_2X}_{\text{group component}}$ the decomposition according to the Price approach yields only a group component, $W = (c_1 + c_2)X$. For a non-social trait ($c_1 \neq 0, c_2 = 0$) the Price approach mistakenly traces group fitness entirely back to a non-existing group effect, whereas contextual analysis correctly assigns group fitness to the individual effect. The fact that contextual analysis handles cross-level by-products correctly in the non-social trait case has led Okasha to conclude that contextual analysis is "on balance preferable" (Okasha, 2006, p. 99) to the Price approach. However, it should be noted that in the soft selection case ($c_1 = -c_2$) contextual analysis decomposes group fitness as

$$W = \underbrace{c_1 X}_{W_{\text{ind}}} - \underbrace{c_1 X}_{W_{\text{gr}}}$$

and hence detects cross-level by-products of magnitude $c_1 X$ even though cross-level by-products are absent since the individual components of fitness sum to zero in each group.

In their study on multilevel selection in water striders, Eldakar et al. (2010) choose contextual analysis for quantifying group selection because contextual analysis controls for "potential cross-level byproducts" (Eldakar et al., 2010; p. 3186). However, as we have seen, contextual analysis does not correctly account for cross-level by-products automatically. Which of the two approaches is correct depends on the kind of individual selection that acts on the system, i.e., the causal structure underlying fitness. In this case, the causal graphs in **Figures 4A,B** both seem possible. Recall that aggressiveness in male water striders is hypothesised to have an effect on the individual component of fitness (aggressive males secure more mating opportunities than non-aggressive males) and on the group component of fitness (patches with higher aggression levels have fewer females). Contextual analysis assumes that the individual component is independent of the group trait: in addition to the group component shared by all males in a group each male has an individual component that is determined by its trait and independent of the group trait. Another, perhaps more plausible, assumption underlies the Price approach: the group trait determines the number of females on a patch and this reproductive resource is distributed to the males according to their competitiveness. We will discuss an experimental intervention that would reveal the correct underlying causal structure in the next section.

3.3. Determining the Preferable Approach

Several authors have discussed the question which of the two approaches is preferable in general (Okasha, 2006; Sober, 2011; McLoone, 2015). However, even the most extensive discussion of this question (Okasha, 2006) has been inconclusive in the sense that in light of the problematic cases discussed above neither can be endorsed unreservedly. We argued that a general preference cannot be justified as the essential difference between the two approaches lies in non-equivalent assumptions about the causal structure of the biological system which, as the problematic cases demonstrate, may be either of the two. However, our reduction of the difference between the Price approach and contextual analysis to a difference between their respective causal graphs has the benefit that experimental interventions that reveal the correct causal structure and with it the correct approach can easily be derived from the causal graphs (Pearl, 2009). Note that while we argue that the suggested interventions in principle reveal the correct structure we do not claim that such interventions are feasible for a given biological system. Moreover, while the two approaches discussed here are the main approaches to measuring the strength of group selection, it may well be possible that neither is suitable in a given scenario. We will discuss this and other limitations of this work below.

Imagine that we have a biological system such as a population of water striders in Eldakar et al. in which intuitive inspection suggests that individual fitness depends on an individual component and a group component as in **Figures 4A,B**. Analysis reveals proposed causal pathways for individual trait and group trait to affect individual fitness via the two components. In particular, such an analysis comprises a hypothesis on the mechanism that mediates the effect of the group trait on the group component of individual fitness. For the water strider example the group trait is mean aggressiveness on a patch, the group component is proportional to the number of females on a patch, and the mechanism that mediates the effect of the former on the latter is female exodus determined by the females' preference for low aggressiveness patches. Choosing contextual analysis or the Price approach for quantification goes hand in hand with the commitment to regard **Figures 4A,B**, respectively, as the causal structure underlying the observed phenomena. The causal structures posited by the two approaches differ in that the Price approach assumes an effect of the group trait on the individual component of fitness. This assumption is reflected in the diagonal arrow in **Figure 4B** that is missing in **Figure 4A**. The two arrows emanating from X in **Figure 4B** represent two distinct cause-effect relations between the group trait and individual fitness. But given the hypothesis on the mechanism that mediates the effect of the group trait on the group component of fitness these two distinct cause-effect relations correspond to two distinct mechanisms through which the group trait affects fitness. Consequently, it is in principle possible to separate the effects by intervening on one of the mechanisms but not the other. This intervention translates to removing the vertical arrows from X to w_{gr} in **Figures 4A,B** so that the system is described by the modified graphs in **Figure 5**. But in the system with suppressed group effects the two causal structures in **Figures 5A,B** can be distinguished on the basis of the observable quantities x, X, and w. In particular, contextual analysis predicts individual fitness to be independent of group membership when the system is being intervened on in this way. The Price approach, however, predicts continued dependence of fitness on group trait due to within-group competition. As these predictions cannot both be true, the intervention allows the identification of one of the two approaches as being in accord with experimental observations.

Corresponding to the causal models expressed in the graphs are mechanisms corresponding to each of the arrows in the model. In the water strider example, given the mechanisms corresponding to the arrows in **Figure 4**, it is now easy to see how a decision for one of the two approaches may be reached. Since the effect of group trait on fitness is mediated by female exodus, the effect can be suppressed by preventing females from leaving patches, i.e., by removing female dispersal between patches (Eldakar et al., 2009). It is crucial that this intervention leaves the diagonal arrow in **Figure 5B** intact. This is because the diagonal arrow represents a different causal pathway, namely the within-patch competition for females which is not affected by preventing females from leaving the patch. An informed decision for contextual analysis can then be reached if fitness is independent of mean aggressiveness on a patch when female dispersal is removed, i.e., if the diagonal arrow in **Figure 5B** was not part of the underlying causal structure in the unperturbed system. The Price approach is more appropriate if fitness still depends on patch composition under this experimental condition.

Both the Price approach and contextual analysis serve the purpose to determine the quantities w_{ind} and w_{gr} in Equation (3), or equivalent quantities (see **Table 1**), from the more easily measurable variables individual trait and individual fitness. In order to achieve this, both approaches require assumptions that can be conveniently represented in terms of causal graphs as in **Figure 4**. We have shown above how, in principle, it is possible to determine which of the two approaches is more appropriate. However, we have seen that the causal structures posited are highly contrived. It seems therefore very well possible that neither of the two approaches is suitable for determining the level-specific strength of selection. This is the case when neither of the causal graphs in **Figures 4A,B** represent the causal structure underlying the biological phenomenon in question.

3.4. Multilevel Selection and Evolutionary Transitions in Individuality

The treatment of multilevel selection in this paper is motivated by the role multilevel selection plays in evolutionary transitions in individuality. When evolutionary units form higher level individuals, selection acts on both levels and the evolution of higher-level function is both facilitated and constrained by lower-level selection (Okasha, 2006; Godfrey-Smith, 2009; Watson and Thies, 2019). The longer-term outcome of selection depends on the interplay between the effects on the individual level and the group level, mediated by population structure. In order to understand how these factors act together, (Okasha, 2006) suggests a diachronic approach to evolutionary transitions that allows regarding the transition of a given system as entity whose parameters change over evolutionary time while it is "in progress." From this perspective, the species of volvocine algae, for instance, that appear to be in between single-celled species and species with faithful reproduction on the group level may be seen to be similar to ancestors of obligately multicellular species, thus offering potential snapshots of the evolutionary history of evolutionary transitions in individuality (Michod, 2007). Indicators of the status of a transition as suggested in Clarke (2016) that rely on the quantification of selection pressures require causally adequate models. The refined causal viewpoint suggested in this paper allows analysis of the drivers of these changes and their dependency on the evolving system itself.

The focus of this paper is on a specific issue regarding the assignment of selection to the levels involved. The composition of groups—while central for their phenotypic properties—is taken for granted, and the models do not include a mechanism of how the groups arise. However, unlike in models of kin selection, groups are formalised as units that partake in causal processes and are assigned properties that may change over evolutionary time. In particular, processes that determine the composition of groups may be subject to selection on multiple levels. Hamilton's relatedness index measures how far the trait distribution within groups deviates from the distribution that would be expected in a randomly mixing population. While the description of population structure in terms of Hamilton's index—like the description of genetic populations in terms of linkage disequilibria—is useful, it captures the notion of a population that is subdivided into groups only indirectly. The causal viewpoint described in this paper acknowledges the levels on which selection acts by promoting groups from environments of individuals to units that themselves interact with a selective environment and thus affect reproduction of their parts in a homogeneous manner. The structure of the population is then a consequence of the mechanisms with which offspring of grouped individuals in a parent generation form groups of individuals in an offspring generation. Since parameters of the mechanisms may evolve, the population structure changes over evolutionary time. Unlike Hamilton's parameter of relatedness that is usually assumed constant for a given system, this perspective of multilevel selection allows modelling adaptations with respect to inheritance on the group level, as required for completed evolutionary transitions in individuality.

The assumption of an additive fitness function in this paper (Equation 1) is required for our argument regarding the difference in causal structure between the two approaches to multilevel selection. However, the distinction between the arenas of selection made by contextual analysis and the Price approach as well as the possibility of a group trait affecting individual selection within groups is of more general interest. Conceptualising interaction partners as part of the selective environment is central for understanding transitions in individuality as social niche construction processes in which individuals evolve to constitute and experience niches in their social environment (Powers et al., 2011; Ryan et al., 2016). During these processes, individual selection that initially acts across a population of loosely interacting individuals becomes increasingly determined by the social environment and thus internal to groups. In a similar vein, Araya-Ajoy et al. (2020) emphasise the role of feedback between selection pressures on traits that determine, for instance, phenotypic response to and impact on the social environment. Since bearers of these traits are not only subject to selection but also constitute the selective environment of their social partners the evolutionary response depends on the interplay between multiple evolving factors. Unravelling these intertwined processes theoretically and empirically requires careful analysis of the causal factors that control them as well as formal tools for their statistical analysis in biological systems. Moreover, the distinction regarding the arena of individual selection made by the two approaches to multilevel selection discussed in this paper seems crucial for understanding the interplay of ecology and evolution that occurs when populations adapt to the (social) environment they themselves create.

Another avenue of research is the role of external ecological factors in evolutionary transitions in individuality. Motivated by the question how selection on an emerging higher level may arise before it attains individual-like properties, Black et al. (2020)

discuss models of selection processes in which external factors scaffold selection on a new level. In these models, ecological factors provide the opportunity for individual cells in patchily distributed groups to determine fitness on the group level by scaffolding a pathway for the evolution of germ-like cells that reproduce the group by dispersal. The resulting division of labour between soma- and germ-like cells relies on the interplay of selection between and within groups. Including many causal factors, as in the models in Araya-Ajoy et al. (2020) and Black et al. (2020), makes it difficult to keep track of the effects they exert on eachother. The causal diagrams discussed in this paper can be extended by external factors and allow the composition of "modules" of interactions between factors. We believe that a causal modelling approach with expressive diagrammatic formalism could be useful not only for the intuitive understanding but also for ensuring consistency and plausibility of these complex models.

We regard the results presented in this paper as first steps of formalising the interaction of selective forces on multiple levels in terms of causal structures. Future work combining these results with social niche construction and other mechanisms of social evolution may examine how causal structures arising in this way are themselves subject to selection and therefore to change over evolutionary time.

4. CONCLUSION

Group selection refines kin selection by splitting individual fitness into two components, i.e., by assuming that fitness is determined by two additional factors that are themselves determined by the variables individual trait and group trait. The causal graphs in **Figure 4** show that this means that group selection adds a layer to the causal structure of selection assumed by kin selection. This addition constitutes a proper refinement of kin selection and corresponds to avoiding averaging over the causes of individual fitness (the "averaging fallacy" described by Sober and Wilson, 1999). From this viewpoint, the tension between contextual analysis and the Price approach can be seen as an instance of the purely formal problem of connecting an additional layer of nodes to an existing graph. The connection schemes proposed by contextual analysis and the Price approach, i.e., the coefficients of the paths targeting w_{ind} and w_{gr} in **Figure 4**, are two solutions to this problem. Since omitted paths in a causal graph represent hypotheses about the absence of effects the correct approach is the approach whose hypotheses are satisfied in the biological system at hand. Phrasing the problem in terms of causal graphs demonstrates that, even in the additive case, other refinements are in principle possible and could apply to scenarios in which the individual component is given neither by soft selection (Price approach) nor by hard selection (contextual analysis) but by intermediate selection regimes (Débarre and Gandon, 2011). Casting an MLS analysis in terms of refinements of causal graphs gives a formal argument for the non-equivalence of MLS and kin selection. We have argued that the refinement introduced by MLS is non-trivial (see difficulties with Price approach and contextual analysis) and provides a view on the system that is tailored to the levels of organisation in the system. This view is crucial when cause-effect relations that pertain to a specific level are manipulated or undergo change, such as during an evolutionary transition in individuality, and the system-level consequences of such alterations are to be predicted. Strengthening the formal core of MLS not only facilitates the application of MLS in evolutionary science but also aids in assessing benefits, limitations, and formal requirements of this approach to empirical and theoretical biological scenarios.

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

CT and RW contributed to conception of the paper. CT wrote the manuscript. Both authors contributed to the article and approved the submitted version.

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REVIEW
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The Major Transitions in Evolution—A Philosophy-of-Science Perspective

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Over the last thirty years, the study of major evolutionary transitions has become a thriving research program within evolutionary biology. In addition to its obvious scientific interest, this research program raises interesting philosophical questions. These fall into two categories: conceptual and ontological. The former category includes questions about what exactly an evolutionary transition is, what form an evolutionary explanation of a transition should take, and whether a general theory that applies to all transitions is possible. The latter category includes questions about the status of the higher-level units to which evolutionary transitions give rise (e.g., organism, superorganism, or individual), and about the nature of the resulting hierarchical organization. Tackling these questions requires an integrative approach that draws on both biology and the philosophy of science.

Keywords: major transitions, evolution, philosophy of science, organism, conflict, cooperation, multi-level selection

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1. INTRODUCTION

The contemporary interest in "major evolutionary transitions" (METs) can be traced to the pioneering works of Buss (1987), Maynard Smith and Szathmáry (1995), and Michod (1999). Though these authors' approaches differed considerably, both in respect of the empirical phenomena they were concerned with and in the type of explanation they sought, they converged on a number of key points. These included: (i) that a series of evolutionary transitions has occurred in the history of life on earth that radically re-shaped subsequent life forms; (ii) that some or all of these transitions involved formerly free-living entities coalescing into larger groups, giving rise to a new level of hierarchical organization; and (iii) that explaining how and why these transitions occured represents an outstanding task for evolutionary biology. In the last twenty-five years many evolutionists have risen to the task, and the study of METs has flourished into a thriving research program, generating much interesting work, empirical and theoretical.

While it is self-evident that METs are of considerable scientific interest, it is perhaps less obvious why they should be of philosophical interest. And yet they are, as attested by the large body of literature on METs authored or co-authored by philosophers of science (Griesemer, 2000; Okasha, 2005, 2006; Godfrey-Smith, 2009; Calcott and Sterelny, 2011; Birch, 2012, 2017; Godfrey-Smith and Kerr, 2013; Clarke, 2014; O'Malley and Powell, 2016; Ryan et al., 2016; Currie, 2019). In this literature we can detect two distinct sorts of philosophical question, which might be called "conceptual" and "ontological," respectively. Examples of the former include questions about how exactly an evolutionary transition should be defined; what form an evolutionary explanation of an MET should take; how concepts such as kin selection, multi-level selection and the "gene's eye view" apply to METs; and whether an overarching theory of evolutionary transitions is possible.

Examples of the latter include questions about what status the new biological units that arise from METs have (e.g., are they organisms, superorganisms, or individuals?); and about the nature of the hierarchical organization that results from the transitions (e.g., is it a hierarchy of parts and wholes? does it have a privileged level or are all levels of equal status?). Tackling these questions requires an integrative approach that draws on both biology and the philosophy of science. The aim of this article is to provide an overview of these questions, to defend particular answers to some of them, and to illustrate by example how philosophical analysis can shed light on this important area of evolutionary biology.

2. CONCEPTUAL QUESTIONS

2.1. What Is an MET?

There is a some disagreement in the literature about what exactly counts as a major evolutionary transition, as a number of commentators have pointed out (Queller, 1997; McShea and Simpson, 2011; Herron, 2021). In their 1995 book, Maynard Smith and Szathmáry offered a 2-fold characterization of an MET. The first was that an MET involves a "change in the way that information is stored and transmitted." In line with this characterization, their list of METs included events such as the transition from RNA to DNA as store of genetic information, and the origin of human language. However, Maynard Smith and Szathmáry also offered a second characterization of an MET, noting that in many cases, "entities that were capable of independent replication before the transition can replicate only as part of a larger whole after it" (p.8). This fits with the idea that an MET is essentially bound up with an increase in hierarchical complexity; that is, it involves the formation of a new higher-level biological unit from a group of lower-level units (Michod, 1999; Bourke, 2011). Consider cases such as the evolution of the eukaryotic cell by the symbiotic union of two prokaryotes; the evolution of multi-cellular eukaryotes from their unicellular ancestors; the evolution of obligate symbioses, e.g., lichens; and the evolution of eusocial insect colonies. In each of these cases, the end result of the transition is that a new higher-level entity exists, built up out of smaller, formerly free-living entities.

There has been considerable debate about which of these characterizations of an MET is "better," and what the relation between them is. [Alternative characterizations have also been suggested, such as that of Robin et al. (2021) who argue that ecosystem effects need to be explicitly included in the definition]. In an update of his views, Szathmáry (2015) offers a spirited defense of the two-dimensional characterization of an MET found in his earlier work with Maynard Smith, arguing that it is a "feature not a bug" (p. 10105). His reason for saying this appears to be the apparent link between changes in how information is stored or transmitted and the evolution of new higher-level units; indeed in many cases the former seems to be a prerequisite for the latter to go to completion. Thus for example, the evolution of multi-cellularity necessitated a system of (within-organism) epigenetic inheritance in which cellular phenotypes could be transmitted across mitosis, thus allowing differentiation and division-of-labor to evolve; while the evolution of eusocial animal societies required a system of signaling and social learning in order for the colony to function as a unit. Thus on Szathmáry's view, the two-dimensional characterization of an MET is not an undesirable ambiguity but has a genuine point, since there is a close empirical link between the two dimensions, despite their conceptual independence.

However, against Szathmáry, others have worried that the notion of a major transition has simply become too broad, sometimes seeming to include any evolutionary event that an author deems "important" enough by whatever yardstick they choose (McShea and Simpson, 2011). My own view is that an MET is best defined in terms of Maynard Smith and Szathmáry's second characterization, that is, as the evolution of a higher-level biological unit out of formerly-free living units. Thus we should set aside the idea that an MET involves a change in how information is transmitted / stored. This means paring the original list of METs to exclude: the origin of the genetic code; the transition from RNA to DNA; the origin of sex; and the origin of human language. This more austere approach seems preferable for three reasons. Firstly, it avoids the murky notion of "the way in which genetic information is transmitted," and sidesteps the question of how exactly changes in mode of information transmission relate to the evolution of higher-level units. Secondly, it offers the best hope that METs will constitute what philosophers call a "natural kind," that is, a set of events (or objects) that are objectively similar to each other as opposed to an arbitrary grouping (Herron, 2021). Thirdly and relatedly, restricting the definition of an MET in this way fits best with the aim of finding a general theory of METs, a hope that animates much of the literature on the topic (e.g., Bourke, 2011; Szathmáry, 2015).

2.2. Complexity and Directionality

Evolutionists have often disagreed on whether the evolutionary process possesses an inherent directionality, leading to certain outcomes with a high degree of predictability. The pre-Darwinian idea that evolution is progressive in the sense of making things "better," e.g., by leading from "lower" to "higher" organisms, has long been abandoned (Ruse, 1996); but vestiges of that idea persist in the widespread notion that evolution by natural selection leads to an increase in complexity, adaptiveness, self-organization, organismic autonomy, or some other quantity (Gould, 2002; Brandon and McShea, 2010). Despite widespread acceptance of the point that natural selection is a brute causal mechanism that lacks foresight, and the recognition that chance events play a crucial role in evolution, the idea that evolution is in some sense directional is still very much alive.

This general issue plays out in an interesting way in relation to the evolutionary transitions. There is a straightforward sense in which an MET leads to an increase in what is sometimes called "vertical" complexity, i.e., degree of hierarchical structuring, since by definition, an MET leads to a new level in the biological hierarchy that was not there before. Given that numerous METs have in fact occurred, it follows that hierarchical complexity, as measured by levels of nesting, has increased over time. However, this obvious point does not settle the question of whether METs are an example of evolution's directionality, for two reasons.

Firstly, it is possible that the increase in hierarchical complexity may have been non-monotone, i.e., there were periods of decline; secondly, since the earliest life forms exhibited the lowest possible level of hierarchical complexity, passive diffusion alone would have led it to increase (Gould, 1988).

The question, then, is whether there is any inherent tendency for METs to occur, that is, for lower-level biological units to form themselves into larger units; and if so, why? The fact that METs have occurred repeatedly—the transition to multi-cellularity alone is believed to have occurred at least sixteen times (King, 2004)—may suggest a positive answer; and the fact that most METs have given rise to functionally complex higher-level units suggests that natural selection, rather than drift and mutation alone, was likely to have played a key role. [In the case of multi-cellularity, the relevant selective pressure may simply have been the survival advantage of being bigger (Bonner, 1988)]. But on the other hand, prokaryotes are the most abundant life-forms on earth, and the vast majority of prokaryotic lineages have not undergone evolutionary transitions, but rather have persisted for long periods of time in something close to their ancestral and ancient form. So the empirical facts do not speak unambiguously either way.

Despite this, a positive answer to this question often seems presupposed in the literature on METs, though it is rarely made explicit. One example of this is the widespread assumption that an MET represents a limit case of the evolution of cooperation / altruism, such that intermediate levels of pro-sociality represent staging posts en route to a full transition (see Birch, 2012 and Bourke, 2011, p.200-1 for critical discussion of this assumption). Thus Stearns (2007) has tentatively suggested that humans may be "stalled" part-way through a major transition from individuals to groups, thanks to intervening conditions. However, caution is needed here. Though it may well be true that the evolution of a new higher-level biological unit is facilitated by the existence of cooperative / altruistic interactions between the smaller units, this does not in itself prove that an evolutionary transition is in any sense an inevitable outcome of the spread of cooperation among smaller units. It is also equally possible that METs are rare singularities that require quite specific ecological conditions and / or fortuitous events (Boomsma, 2009), and that intermediate levels of cooperation among smaller units are evolutionarily stable, rather than being staging posts en route to a transition (Herron et al., 2013). The indisputable utility of the principles of social evolution in helping us to understand the evolutionary pressures at work in an MET should not seduce us into assuming that there is an inherent tendency for high levels of pro-sociality to lead to an MET. It may well be that an MET represents something qualitatively different from the evolution of altruism.

The underlying problem here, I suggest, is parallel to one that arises in other discussions of evolutionary directionality, namely that it is not entirely clear how to operationalize the thesis that there is an inherent tendency for METs to occur and thus an inherent tendency for vertical complexity to increase. It is not obvious what empirical data, even if we had it, would settle this question. Even if vertical complexity could be measured unambiguously, any observed trend is compatible with the hypothesis of an inherent tendency toward increase or with the opposite hypothesis, so long as "constraints" and "counterveiling forces" can be invoked. Hypotheses about evolutionary directionality thus suffer from a severe form of what philosophers call "underdetermination by the data." However, we need not despair entirely. Some progress on the question could be made if a well-established "theory of evolutionary transitions," of the sort envisaged by Szathmáry (2015), were developed. Such a theory could plausibly help to identify the relevant selective pressures and ecological conditions that push a biological system toward an MET, and could help resolve the question of whether an MET is a predictable, or at least somewhat likely, outcome in any biological system characterized by a high degree of cooperative or altruistic interactions.

2.3. A General Theory?

Should we hope for an overarching theory that can explain all the known METs? Or should we be content with a series of piecemeal explanations? The answer to this question depends on two things. The first is the extent to which the different evolutionary events that we call "METs" are objectively similar, or constitute a natural kind; for if they do not, then it would be misplaced to seek a general theory. The second is whether, even if the METs do constitute a natural kind, a common set of explanatory principles can be identified that applies to them all. These two issues are related but distinct.

So long as we define an MET in the way recommended above, as the evolution of a higher-level biological unit from smaller, formerly free-living units, the objective similarity requirement seems likely to be met, at least to a reasonable degree. Most evolutionary events do not involve the formation of new higher-level units; so singling out the ones that do, and co-classifying them, surely picks out a genuine kind. Obviously there are still differences between the METs; no two evolutionary events are going to be similar in all respects. Queller's distinction between "fraternal" and "egalitarian" transitions is relevant here; in fraternal cases, the lower-level units that form a larger unit are themselves closely related (e.g., single-celled to multi-celled eukaryotes); while in the egalitarian cases, the lower-level units are unrelated and may be from different species (e.g., the union of two prokaryotic cells into a eukaryotic cell) (Queller, 1997, 2000). This is an important distinction, as different evolutionary pressures will apply in each case; but it is still compatible with METs being a natural kind composed of two sub-kinds. This at least seems like a plausible working hypothesis.

Could a common set of principles explain all of the transitions? This is a trickier issue. Since by definition, all METs involve "the same" thing, namely the formation of higher-level units from collections of smaller units; and since natural selection was presumably implicated in this, it is tempting to assume that basic Darwinian principles will illuminate the METs. And to an extent they do, as a number of authors have noted (Maynard Smith and Szathmáry, 1995; Bourke, 2011). For example, we know that there must have been a short-term selective advantage to the smaller units in order for them to form a collective; that the collective would not necessarily be stable owing to defectors pursuing their own interests; that mechanisms for aligning the interests of the smaller units (e.g., kinship, policing, division-of-labor) could alleviate this problem (Frank, 2003); and that higher-level selection (between collectives) would need to trump lower-level selection (between units within a collective) in order for the collective to evolve adaptations of its own (Michod, 1999). These and other principles, stemming from general evolutionary theory, are likely to be relevant to all METs.

However—and this is why the issue is tricky—"relevant" is not the same as "useful" nor "explanatorily fundamental." Explanations of METs at this level of abstraction, while not wrong, may not tell us what we want to know. Consider for example the evolution of the first proto-cell, the first eukaryotic cell, and the first eusocial insect colony. These events do have something in common, but it may be that focusing on the commonality obscures, or at least does not help answer, important biological questions. A full understanding of any one of these transitions requires a detailed description of the sequence of actual stages involved, not just an abstract analysis of the evolutionary forces at work. This, in turn, reflects the fact that explanations in terms of evolutionary advantage, while important, are not the only sorts of explanation in biology (hence the widely-appreciated need to integrate the study of function with the study of mechanism). In the case of the proto-cell, for example, we certainly want to know why it was advantageous for replicating molecules to become compartmentalized; but we also want to know how the compartments were formed; what their structure was; what the sequence of stages was that led to compartmentalization; and how and why one stage evolved into another. The answers to these questions may well be specific to the origin of the proto-cell, and will not necessarily have close analogs in the other METs.

Relatedly, there is a risk when studying METs of over-emphasizing the similarities between different transitions, or simply assuming ahead of time that they can all be explained in the same way. This is not a hypothetical point, since there has been a certain tendency to over-apply the social evolution framework (or simple models belonging to that framework such as the prisoner's dilemma.) Thus for example, Rainey et al. (2014), in an article entitled "Microbes are not bound by sociobiology," argue persuasively that terms and concepts from social evolution theory, such as "cheating" and "public good," have been misappropriated in studies of bacterial sociality with harmful consequences. In particular, Rainey et al. (2014) object to the practice of using the term "public good" to refer to any extracellular metabolite secreted by a microbe, irrespective of whether it is actually costly to produce or beneficial to others. An *a priori* commitment to the idea that a collective action problem is central to every MET appears to be the source of this confusing practice.

In short, there are clear thematic commonalities among the various METs, and we can point to general evolutionary principles that likely apply in all cases (though in light of the Rainey's point above, we should take care not to pre-judge this). But precisely because of their generality, such principles will yield rather coarse grained explanations. The real issue, therefore, is not whether a general theory of METs of some sort can be found, but whether the principles of such a theory could be suitably general to apply to all METs *and* suitably specific to yield explanations at the right "grain" to answer the biological questions that interest us. The jury is still out on this question.

2.4. Hierarchical vs. Genic Explanations

A number of authors have pointed to a distinction between "genic" and "hierarchical" approaches to the METs (Buss, 1987; Queller, 1997). On the genic side, we find authors such as Maynard Smith and Szathmáry (1995), Bourke (2011, 2014), and West et al. (2015); on the hierarchical side, we find Buss (1987), Michod (1999) and Szathmáry (2015). The distinction is one of preferred explanatory approach and / or conceptual toolkit. The genic approach is reductionistic in spirit, borne of the general conviction that Darwinian evolution should be explained in terms of direct selective advantage to individual replicators. Applied to the METs, this suggests that the key thing we need to understand is what the selective advantage to the lower-level units was from forming a larger unit. The hierarchical approach, by contrast, is anti-reductionistic, emphasizing emergent properties of wholes and the role of multi-level selection in driving evolutionary outcomes. To explain an MET, on this approach, we need to understand why selection between higher-level units was able to dominate selection within them, thus allowing higher-level units to evolve into functionally integrated units.

In his review of Maynard Smith and Szathmáry's (1995) book, Queller (1997) argued that we need not choose between the genic and hierarchical approaches—"we can, indeed must, have it both ways" (p. 187). Queller argued that Buss's "failure to do his genetic sums" had led him to questionable conclusions about the evolution of multi-cellularity, but that the hierarchical approach nonetheless "leads to the right questions," such as what an organism is (p.187). Queller is surely right that the genic vs. hierarchical issue is something of a false dichotomy, since there is no obvious respect in which the two are incompatible; and in general, a pluralism of explanatory schemes is often a good thing in science (Birch and Okasha, 2015). However, we should distinguish between two different ways of "having it both ways," that is, of trying to combine genic and hierarchical approaches to the METs.

The first way is to adopt the genic approach for some METs and the hierarchical approach for others. Consider again the distinction between fraternal and egalitation transitions. Though Queller (1997) does not suggest this, a natural idea is that fraternal transitions are best explained using the genic approach and egalitarian transitions using the hierarchical approach. For the hallmark of a fraternal transition is the high relatedness between the lower-level units, and the consequent potential for kin selection to operate. Thus in the transition to multi-cellularity, for example, one well-known scenario posits a proto-group of cells that arose from the products of mitosis remaining physically attached to their parent cell; since its constituent cells would then be clonally related, simple kin selection logic then explains how the proto-group could evolve into a cooperative unit. By contrast, in an egalitarian transition, such as the formation of the eukayortic cell by the symbiotic union of unrelated prokaryotes, a different sort of explanation is needed; kin selection cannot be part of the story (Bourke, 2011). Plausibly, the explanation will appeal to the group-level advantage from combining replicating units of different sorts in a single group. Such an explanation is broadly "hierarchical," in the sense that it appeals essentially to emergent or group-level properties; and it explains the transition by invoking a between-group selective process.

The second way of trying to reconcile the two approaches is different. Rather than applying the genic approach to some METs and the hierarchical approach to others, perhaps one and the same MET can be explained using either approach? This is a reconciliation of a different sort; it ties in with the broader idea, familiar since Dawkins (1976) "necker cube" analogy, that a single evolutionary process may usefully be viewed from multiple perspectives. Since in an MET, the higher-level unit comes to be functionally organized thanks to the alignment of the fitness interests of the constituent gene-level sub-units (Bourke, 2014), it stands to reason that the MET can be viewed from either a genic or hierarchical perspective. In support of this second reconciliation, we should note that even in a fraternal transition, where the genic approach is most natural, the end result is still an increase in hierarchical complexity; and even in an egalitarian transition, where the hierarchical approach is most natural, it remains true that without a direct selective advantage to each of the (unrelated) lower-level replicators, they would never have voluntarily entered into a group-living arrangement in the first place. Thus elements of both explanatory frameworks, genic and hierarchical, do seem applicable to all METSs.

This second way of reconciling the genic and hierarchical approaches to the METs might be regarded as a special case of the well-known idea that inclusive fitness and multi-level selection are "equivalent" formulations of social evolution theory, despite having been pitted against each other in the past. This "equivalence thesis" has been widely endorsed in the social evolution literature, though with some dissenters. [Supporters of the equivalence thesis include Queller (1992), Frank (1998, 2013), Kerr and Godfrey-Smith (2002), Lehmann et al. (2007), Marshall (2011); dissenters include Hölldobler and Wilson (2009), van Veelen (2009), Nowak et al. (2010), Traulsen (2010)]. Since the genic approach is intimately bound up with inclusive fitness, and the hierarchical approach with multi-level selection, it is tempting to regard the equivalence thesis as supplying a theoretical underpinning for the idea that any MET can in principle be explained using either a genic or a hierarchical approach.

However, some care is needed here. It is true that there is a *formal* equivalence between inclusive fitness and multi-level selection, in the sense that in *some* models for the evolution of a pro-social behavior, it is possible do the evolutionary analysis in either multi-level terms, by partitioning fitness variation into within-group and between-group components, or in inclusive fitness terms, by partitioning fitness into direct and indirect components. However it is unclear whether this is true of all models, in part because the equivalence results rely on approximations including weak selection (Lehmann and Rousset, 2014); and in part because the multi-level analysis presupposes the existence of group structure while the inclusive fitness analysis does not (Birch and Okasha, 2015). Moreover, formal equivalence is not the same as causal-explanatory equivalence (Okasha, 2016). For the aim of evolutionary analysis is not simply to predict the outcome of evolution, or to compute a correct expression for allele frequency change, but rather to produce causal explanations. One of two "formally equivalent" approaches may yield a better causal representation of the evolutionary processes at work in a particular biological system. So it would be overhasty to conclude, from the formal equivalence results alone, that inclusive fitness and multi-level selection are necessarily equivalent in respect of their causal adequacy; and by the same token, we cannot conclude that genic and hierarchical approaches to the METs are always of equal explanatory power. The equivalence thesis, therefore, does not discriminate between the two ways of reconciling the genic and hierarchical approaches.

To conclude, Queller's idea that the genic and hierarchical approaches both have something to contribute to the study of METs is correct. But there is more than one way of trying to effect a reconciliation between the two approaches. It may yet turn out that the genic approach is better suited to studying some METs while the hierarchical approach to others; alternatively, it may turn out that any MET can be fruitfully studied using either approach.

2.5. METs and the Levels of Selection

It is interesting to consider how the study of METs relates to the traditional "levels of selection" (or "units of selection") discussion in evolutionary biology. The latter discussion traces to Darwin (1859) and achieved prominence in the second half of the 20th century though works by Williams (1966), Lewontin (1970), Hamilton (1975) and Dawkins (1976, 1982), among others; it thus pre-dates the contemporary work on METs. Yet clearly there is a conceptual connection between METs and levels of selection. For in an MET, formerly free-living individuals become integrated into a larger unit; so there is the potential for natural selection to act at two different levels (Okasha, 2005; Wilson, 2010). As Buss (1987) first emphasized, for an evolutionary transition to be successful, it is necessary for higher-level selection to "trump" lower-level selection; typically this requires the evolution of mechanisms, such as policing, to regulate the selfish tendencies of the lower-level units and / or to align their evolutionary interests. Thus it is unsurprising to find that many themes from the traditional levels of selection discussion, such as altruism versus selfishness, individual versus group interests, and the importance of genetic relatedness, re-appear in the literature on METs.

In some ways, the MET research program has breathed new life into the levels of selection discussion. Some biologists have wanted to dismiss the traditional levels discussion as a storm in a teacup, arguing that in practice, individual selection is all that really matters in biology, whatever about other theoretical possibilities. [Thus for example, Waddington (1975) described the debate over group selection as "a rather foolish controversy"]. Others have dismissed the debate as largely semantic. But in the light of the METs, these dismissive attitudes are hard to defend, for two reasons. Firstly, group selection appears implicated in many if not most of the METs (though a kin selection / inclusive fitness perspective may be equally valid in some of these cases, as discussed above). Given that METs clearly have occurred, the evolutionary importance of group (or multi-level) selection can hardly be denied. Secondly and more importantly, the METs remind us that even paradigm biological individuals can be regarded as groups or collectives, once we take a sufficient long time-horizon. Multi-celled organisms and eukaryotic cells, for example, are both collective entities that evolved out of smaller free-living biological units. Thus once we adopt the expanded evolutionary perspective that is necessary to understand the METs, the view that "individual selection is all that matters in practice" clearly cannot be sustained; and indeed the very notion of an "individual" is called into question.

Despite their conceptual kinship, there is one important difference between the study of METs and the traditional levels of selection debate. The latter debate dealt with selection and adaptation at *pre-existing* hierarchical levels, and largely set aside the question of the origin of the biological hierarchy itself (Griesemer, 2000; Okasha, 2005). Thus consider for example how Lewontin (1970) formulated the "units of selection" question in his highly influential article. Lewontin began by observing that there are three essential requirements for the process of evolution by natural selection: variation, associated differences in fitness, and heredity. He then observed that in principle, biological units at various hierarchical levels, above and below that of the individual organism, could satisfy these three requirements. Thus for Lewontin, the "units of selection" question stemmed from two factors: (i) the abstract nature of the fundamental Darwinian requirements; and (ii) the fact that biological units form a nested hierarchy. There is nothing wrong with Lewontin's formulation per se, but it is incomplete in one important respect. For clearly, hierarchical organization is not simply a brute fact about the living world: the earliest life-forms were not hierarchically complex. Thus there must be an evolutionary story to be told about how the biological hierarchy evolved in the first place; and the METs form a key part of that story.

This implies that the link between METs and the traditional levels of selection issue is more complex than it seems; it is not just a case of themes from one discussion re-appearing in the other. One useful way to look at it is the following. In the traditional levels discussion, hierarchical organization is treated as "exogenous," something that is part of the assumed background against which evolutionary explanations are constructed but does not receive any explanation itself (Okasha, 2021). In the literature on METs, by contrast, hierarchical organization is "endogenized," since the aim is precisely to offer an evolutionary account of how biological units came to form a nested hierarchy in the first place. In philosophical terms, hierarchical organization thus moves from being part of the *explanans* to being part of the *explanandum*. Looked at this way, we have an instance of a common pattern in evolutionary biology, in which features that were once part of the assumed biological background against which evolutionary explanations take place (e.g., sexual reproduction, gamete dimorphism, and fair meiosis) are progressively endogenized as the science advances, leading to an increase in theoretical generality (Okasha, 2021).

Finally, the study of METs forces us to re-examine certain issues from the traditional levels of selection discussion. One such issue is how best to formulate the basic Darwinian principles. Dawkins (1976, 1982) introduced the concepts of "replicator" and "vehicle" as part of his abstract analysis of the elements of Darwinian evolution; Hull (1980) later suggested the term "interactor" in lieu of vehicle. Though these concepts have their merits, and certainly clarified aspects of the traditional levels debate, they arguably lack generality, for they help themselves to something that needs to be explained. Thus Dawkins characterized a replicator as an entity with high "copying fidelity" that passes on its structure intact to its descendants; while Hull characterized an interactor as an entity that "interacts as a cohesive whole with its environment." However, recent work on METs teaches us that high copying fidelity and organismic cohesion are evolved attributes. The first replicators would have had low copying fidelity, as the mechanisms that ensure copying fidelity did not then exist [This is the source of "Eigen's paradox" as discussed by Maynard Smith and Szathmáry (1995)]. Similarly, the first multi-celled organisms were unlikely to have been particularly cohesive, as they were probably mere clusters of cells that arose when daughter cells failed to fully separate from their parents, and thus would have lacked any group-level functional organization. If we want evolutionary theory to be able to explain how attributes such as high-fidelity replication and organismic cohesion evolved initially, we had better not build these attributes into the concepts that we use to formulate the basic Darwinian principles. This is another conceptual moral of the METs.

3. ONTOLOGICAL ISSUES

The term "ontology" has a dual meaning in philosophy. It can refer to the traditional sub-branch of philosophy that tries to answer the question "what things exist in the world?" It can also refer to the objects of study of some particular field of enquiry, as when we say that electrons belong to the "ontology of particle physics," for example. It is this second sense of the term that is relevant here. The study of METs raises interesting questions concerning the ontology of evolutionary biology; these have to do with the nature of biological entities, hierarchical organization, and part-whole structure.

3.1. Hierarchical Organization

It is a commonplace that the entities studied in biology vary greatly in size, from biomolecules at one extreme to ecosystems at the other, and that these entities form a hierarchy of sorts, with larger ones composed of smaller ones. This observation long predates the MET research program, of course. Though there have been attempts to theorize systematically about hierarchical organization (e.g., Salthe, 1985), most descriptions of the biological hierarchy are fairly casual. A typical description is: "gene–chromosome–cell–tissue–organ–organelle–multi-celled organism–kin group–colony–ecosystem." Clearly there is something right about this description, but it raises a number of awkward questions. What is the criterion for being a level in this hierarchy? Where do species and clades fit in? Is there a unique biological relation that relates entities at adjacent levels (e.g., gene to chromosome and organism to kin group)? Is it a problem that some entities in the above list (e.g., organisms) are functionally organized but others (e.g., ecosystems) are not? Should we think of the hierarchy as one of (what philosophers call) part-whole inclusion? (That is, do the larger entities contain the smaller ones as their "parts" in the same way that other macroscopic objects, e.g., cars, contain parts)? Do all biological entities belong to a single hierarchy?

Some progress with these questions was made by Eldredge (1985), who argued that there are in fact *two* biological hierarchies: ecological and genealogical. In the former, the relation that "binds" a number of lower-level units into a single higher-level unit is ecological interaction, while in the latter it is genealogical relatedness. Thus, entities such as species and monophyletic clades belong in the genealogical hierarchy, while entities, such as colonies and social groups belong in the ecological hierarchy. Interestingly, Eldredge argues that a multi-celled organism belongs in both hierarchies (and is the only entity that does); the reason is that the cells within a single organism are clonally derived from a single zygote, so form a monophyletic group of cells; and they also interact ecologically, for example via cell-cell signaling, and have a common fate. Thus organisms stand at the intersection of the genealogical and ecological hierarchies.

The study of METs brings further clarity to the nature of hierarchical organization in biology. As emphasized above, an MET by definition gives rise to an entity at a previously unoccupied hierarchical level, so can be thought of as a means by which *some* of the hierarchical structuring in the biota evolved. The qualification "some" is needed for two reasons. Firstly, the hierarchical organization that results from an MET corresponds to Eldredge's ecological hierarchy, not his genealogical hierarchy. This point is obvious when we consider the egalitarian transitions, whose hallmark is precisely that higher-level units are formed out of unrelated smaller units. It is also obvious once we note that entities such as species and clades are not the product of METs. Secondly and less obviously, even once entities belonging to the genealogical hierarchy are excluded, the hierarchy that results from METs is more restricted than that given in some descriptions of "the" biological hierarchy, such as the illustrative one at the start of this section. In particular, tissues and organs do not belong; for although they are composed out of cells, they did not evolve by groups of free-living cells combining themselves into a larger unit, but by differentiation among the parts of an already existing multi-celled organism. In short, to belong in the hierarchy that results from the METs, an entity needs to be "homologous with organisms in a free-living state, either extant or extinct," to borrow a phrase of Dan McShea (2001); this explains why organelles and cells belong, but tissues and organs do not. This is not to deny that there could be a genuine point to a characterization of the biological hierarchy that includes organs and tissues as levels. But it is important to see that the logical basis for such a characterization, i.e., the implicit criterion for what counts as a level, would be quite different from that of the hierarchy that results from the METs.

The point that the METs account for some but not all of the part-whole structure in the living world, combined with Eldredge's distinction, helps us toward a better understanding of, and a more principled way of describing, hierarchical organization in biology. It does not resolve all the questions though. One that remains concerns the existence or otherwise of a privileged hierarchical level.

3.2. A Privileged Level?

There are two ways of thinking about the hierarchical structure that results from the METs. According to the first way, entities occupying different levels differ in their vertical complexity, but apart from that there is nothing that distinguishes one level from other. The levels thus enjoy an equal ontological status, none being privileged over any other. According to the second way, the levels do not have equal status; rather, the highest level occupied, in any particular biological system, is privileged over all the lower levels. For only entities at the highest level count as "organisms" (or perhaps: "evolutionary individuals") entities at lower levels relinquished that status when the transition took place. Thus a mitochondrion and a cell in a modern metazoan, for example, have a fundamentally different status from the metazoan itself (presuming that the metazoan is not part of an integrated colony that has itself resulted from an MET).

The first view is implicit in much of the traditional levels of selection discussion, discussed above (e.g., Lewontin, 1970). A key idea in that literature is that it is a mistake to focus exclusively on "the individual" when thinking about how Darwinian evolution works; natural selection can operate at other levels too, e.g., the group level, given that the fundamental requirements—variation, heredity, and multiplication—are satisfied by entities above and below that of the individual. This idea fits naturally with the view that the different levels in the biological hierarchy are on a par. The second view, by contrast, is implicit in the idea that an MET involves a change in the "level of individuality," a characterization favored by certain theorists of the METs (e.g., Michod, 1999). According to this idea, during an MET the lower-level units relinquish their status as individuals, for they give up their free-living existence and evolve into parts of a larger unit; that larger unit then counts as a new, higher-level "evolutionary individual." Thus, the attribute of "individuality" is lost by the smaller units but gained by the larger unit. This fits with the idea that the hierarchy generated by METs does have a privileged level, namely the highest occupied level in a given system.

Which of these views is preferable? It depends in part on the vexed question of what "individuality" is; this is a topic of much recent discussion (Pradeu, 2016). My own view is that a version of the second view is defensible, but that it is best expressed in terms of the concept of an *organism*, rather than that of an "evolutionary individual"; where an organism, roughly speaking, is an entity that exhibits adaptations, is functionally organized, and exhibits a certain "autonomy" or "agency." That is, what happens during an MET is that there is a shift in the level of "organismality" entities that were formerly organisms lose that status, and a new organism evolves that is made up of parts that are homologous to the original, smaller organisms. Thus, there is indeed a privileged level in the hierarchy that results from the METs, namely the highest occupied level, for it is there and only there that we find organisms.

This version of the second view may seem hard to square with the popular idea that some METs have given rise to *superorganisms*, such as eusocial insect colonies. If this is true, surely there cannot be anything privileged about the organismic level in the hierarchy generated by the METs, and surely we cannot equate the organismic level with the highest occupied level? However, an important argument of Queller (1997) deserves mention here. Queller argues that the very notion of a superorganism should be rejected on logical grounds. If an entity, such as a honey bee colony, exhibits sufficient functional organization and a sufficiently low level of internal conflict to merit being called a superorganism, it is more consistent to simply describe it as an organism. As Queller puts it, "we designate something as an organism, not because it is *n* steps up on the ladder of life, but because it is a consolidated unit of design" (1997, p.187). If Queller is right about this, as I believe that he is, it follows that we can treat the organismic level as ontologically privileged in the hierarchy generated by the METs without falling into contradiction.

Finally, it is interesting to note that the issue here—privileged level or not—also arises in relation to the genealogical hierarchy, but in reverse form. The lowest level in the genealogical hierarchy is usually taken to be the species; the entities at higher levels are monophyletic clades of various sizes (traditionally assigned to different "ranks," though these have been abandoned by modern "rank-free" approaches to phylogenetic systematics). Biologists concerned with classification and systematics have long disagreed about whether species have a different ontological status to higher taxa or not. One traditional view is that species are "real" biological units whereas higher taxa are merely "conventional;" this implies that the species level is a privileged level in the genealogical hierarchy. But an alternative view holds that species are merely the "basal taxonomic units;" that the concept of monophyly can apply at the species level; and that all monophyletic clades, including species, are equally "real." Here is not the place to try to resolve this (somewhat murky) issue; the point to note is simply the analogy with the parallel issue concerning the hierarchy that results from the METs.

3.3. METs and Part-Whole Structure

Our final ontological issue follows directly from the previous one; it concerns the nature of the part-whole structure (or hierarchical organization) to which METs give rise. Consider again the idea that an MET involves a higher-level entity gaining organismic status and lower-level entities giving up that status. If this conceptualization is right, it suggests that an organism cannot have parts that are themselves organisms; this is an example of what philosophers call an "exclusion principle." Indeed this principle follows directly from the definition of an organism found in Queller and Strassmann (2009), which is motivated by their study of METs. They define an organism as "the *largest* unit of near-unanimous design," where the "unanimity" of a biological unit means that its constituent parts exhibit a lot of cooperation but little conflict (p. 3144, my emphasis). As Godfrey-Smith (2009) notes, Queller and Strassman's definition implies that if an entity is an organism, any parts or sub-units that it contains are not organisms.

Is the part-whole exclusion principle plausible? In many cases it is. An amoeba is clearly an organism; but the chromosomes and mitochondria that it contains are not. A metazoan is clearly an organism; but the cells within it are surely not. However, in other cases the principle seems less obviously true. Think for example of the gut bacteria in each of us; they are clearly organisms, and one might well think that they are parts of us. Or think of one of the partners in an obligate symbiosis, such as the fungal partner in a lichen, for example. On the face of it, there is nothing obviously wrong with the idea that the lichen itself and the fungus are both organisms, the latter being part of the former. Finally, consider a honey bee colony. There is a strong case for regarding the whole colony as an organism, as noted above; but it seems counter-intuitive to say that the individual bees are not organisms, as the exclusion principle would require. [Indeed as Godfrey-Smith (2009) notes, Queller and Strassmann (2009) say in one place that the individual bees *are* organisms, thus, implicitly contradicting their own definition].

How should we resolve this issue? There are three possible options. The first is to simply reject the exclusion principle, i.e., to claim that some *bona fide* organisms do contain other organisms as parts, even if most do not. I see no decisive objection to this; though it is interesting to note that according to a venerable philosophical tradition, there is an important sub-category of natural kind terms, known as *sortals* or *substance sortals*, that are thought to satisfy the part-whole exclusion principle (Grandy and Freund, 2021). (The sortal to which an entity belongs is to meant to tell us the "fundamental sort" of thing it is, and to settle questions about the entity's identity and persistence over time). If the term "organism" in evolutionary biology violates that principle, despite functioning much like a sortal term in other respects, this would be a philosophically significant finding. The second option is to retain the exclusion principle and try to explain away the apparent counterexamples. Thus in the lichen example, we would need to argue either that the lichen is not a single organism or that its fungal partner is not; and similarly for the honey-bee colony. One possible motivation for this view is the idea that in both these cases, the MET has not gone to completion (and may never do so). That is, the lichen and the honey bee colony do not count as organisms, precisely *because* their constituent parts have not fully relinquished their organismic status, in the way that the cells of a metazoan, or the mitochondria of a eukaryotic cell, have done.

The third possible option is to argue that being an organism is a matter of degree, not an all-or-nothing matter. This is quite plausible, given the gradualness of evolution. Even if an MET does produce a new higher-level entity that is clearly an organism, there will likely be a transitional phase, or gray area, when the entity's organismic status is moot. Moreover, as noted in section 2.4, such a phase could reflect a stable equilibrium, so is not necessarily a temporary staging post en route to a "full" transition. This option opens the door to arguing that some entities, such as lichens and honey bee colonies, are partly though not wholly organismic. Also, this allows a modified version of the part-whole exclusion principle to be retained, restricted to entities that are fully organismic. The modified principle thus says that no entity that enjoys *full* organismic status can have parts that also enjoy that status.

The choice between these three options raises difficult issues, both philosophical and scientific. (The main scientific issue is how we should understand the concept of organism in the light of the METs; the main philosophical issue is whether or not we should treat the part-whole exclusion principle for organisms as sacrosanct, and why). My own view that is that the second and third options are both defensible, and on balance preferable to the first option; but I know of no consideration for or against any of the three options that strikes me as decisive.

4. CONCLUSION

Evolutionary biology has long been a source of fascination for philosophers. There are two main reasons for this. The first is that evolutionary biology promises to shed light on topics of perennial philosophical interest, such as human nature, altruistic vs. selfish behavior, and the tension between individual self-interest and group welfare. The second is the prevalence of conceptual issues within evolutionary biology that are ripe for philosophical analysis, such as the rationale for using purposive language, the nature of biological classification, and the relation between proximate and ultimate explanations.

Set against this background, it is unsurprising that recent philosophers of biology have turned their attention to the study of METs, given their evident scientific importance. However to a practicing biologist, it may not be obvious why philosophers think they have something to contribute to this area. The aim of this article has been to explain why this is so. We have distinguished two types of philosophical question—conceptual and ontological—that are thrown up by the MET research program; we have identified a number of questions of each type and suggested answers to some of them. While these answers are inevitably provisional, my hope is that the reasoning behind them illustrates the potential for fruitful interplay between philosophers and biologists working in this area.

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SO carried out the research and wrote the article.

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Distributed Adaptations: Can a Species Be Adapted While No Single Individual Carries the Adaptation?

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Species' adaptation to their environments occurs *via* a range of mechanisms of adaptation. These include genetic adaptations as well as non-traditional inheritance mechanisms such as learned behaviors, niche construction, epigenetics, horizontal gene transfer, and alteration of the composition of a host's associated microbiome. We propose to supplement these with another modality of eco-evolutionary dynamics: cases in which adaptation to the environment occurs *via* what may be called a "distributed adaptation," in which the adaptation is not conferred *via* something carried by an individual of the adapted species (as with genes, behavior, or associated microbes), but by some structural or compositional aspect of the population. Put differently, the adaptively relevant information cannot be reduced to information possessed by a single individual, whether genetic or otherwise. Rather, the adaptively relevant information is distributed, and is found strictly at the population level. While human culture is presumably such a case, as may be cases found in social insects, we want to suggest that there are other cases that belong to this category and to explore its evolutionary implications. In particular, we discuss the factors that affect whether adaptive information is stored in a distributed way, to what degree, and what kinds of adaptive information are most likely to be found in this modality of adaptation.

Keywords: adaptation, major transitions, cultural evolution, social learning, collective behavior, collective memory, collective decision making, information theory

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1. DISTRIBUTED ADAPTATIONS

Can a species be adapted to its environment in a certain respect, while no single individual carries the adaptation? Consider the following example: imagine a species in which individuals need to find suitable habitat to breed or dwell when they reach maturity. Adaptations that are carried by the individual that could accommodate this need are, for example, innate knowledge about the environmental cues that characterize a suitable site or learned knowledge about where such sites are to be found. These solutions consist of *individual-level adaptive information*. Alternatively, a *distributed adaptation* (DA), comprising *population-level adaptive information*, might accommodate the need as well: individuals could rely solely on searching for other individuals of the species, and joining them. Such a strategy is likely to be adaptive, since—by definition—locations in which multiple individuals are found are highly likely to be habitable (see, e.g., Bee, 2007; Fouquet et al., 2021). In this case, the information necessary for survival is encapsulated in the species' demography as a whole, i.e., in the spatial distribution of the species' individuals (coupled with their aggregating behavior).

Our discussion will proceed as follows. In section 2 we define and develop the concept of distributed adaptations. In section 3 we discuss the concept's relation to other phenomena and paradigms, and in section 4 we offer a number of comments regarding its utility and some theoretical implications. Section 5 summarizes and offers a series of concluding remarks and open avenues for further exploration.

2. THE EVOLUTION OF DISTRIBUTED ADAPTATIONS AND THEIR ECO-EVOLUTIONARY IMPLICATIONS

To discuss modalities of adaptation, the definition of an *adaptation* must be clear. As an intuitive and broad definition which does not pre-determine the level at which an adaptation is implemented, we will use the term adaptation to refer to any trait that facilitates survival and persistence in the environment, including over multi-generational timescales.

To illustrate the notion of a distributed adaptation we discuss two hypothetical cases. Let us consider first the example from above in more detail: a species of frogs living in a semi-arid region. Let us imagine that an individual must find a seasonal pond in which to survive and reproduce, but these ponds are scattered across inhospitable terrain (see, e.g., Goldber et al., 2009; Degani, 2016, 2015). Searching haphazardly for them is costly. A possible adaptation might involve some level of innate knowledge of the terrain or navigation skills, such as having preferences to go in certain directions. But this may not be possible if the location of ponds is highly irregular or changes too quickly (e.g., Vaira, 2005). An alternative adaptation involves learning the location of ponds from the mother, but this may not be possible in a species in which there is little or no parental care after hatching. A third possibility would involve some cognitive and sensory capabilities that improve navigation to water or to the individual's specific natal environment. Such adaptations are possibly difficult to evolve or may be costly to maintain. A distributed adaptation to the problem of finding ponds may be simple: the frog listens to croaking sounds coming from conspecifics and navigates toward them. Since the sounds of several other individuals coming from the same location typically indicate hospitable locations, going in the direction of croaking will often lead in the direction of a pond. The sensory and behavioral adaptation is simple and may build on existing sensory abilities and behavioral preferences (Bee, 2007; Fouquet et al., 2021). Navigating toward sounds of multiple conspecifics thus constitutes an adaptation to a major challenge posed by the species' environment.

This hypothetical case highlights the essence of what we term distributed adaptations: the information that the frog relies on to find the ponds is not "stored" in any individual. It is, for example, impossible for a single frog to learn from another where to find the ponds, nor does any frog remember the location of ponds across the terrain. The information about the location is honestly represented by the actual location of the frogs in the population, and croaking makes this distributed information accessible. The adaptive *information* can thus be said to be *distributed information*. A possible implication of such a scenario, for example, may be that the distributed adaptation is a potential source of difficulty in re-introduction programs for species that had gone locally extinct. Simply reintroducing enough individuals may not be sufficient, if they are not spread out in a way that realizes the appropriate distribution of the population in its specific terrain. It would be critical in such a case to realize that the adaptation to the environment is a distributed adaptation, to allow wildlife managers to plan reintroduction programs accordingly. Considering only the individual-level adaptations that allow the frogs to persist in their environment misses an important aspect of the adaptation.

This example illustrates how distributed adaptation differs from related theoretical notions, in particular division of labor (Robinson, 1992; Beshers and Fewell, 2001) and niche construction (Laland et al., 2000, 1999; Odling-Smee et al., 2003, 1996). There is no division of labor in the frog population, with some frogs better able to navigate than others, leading to group benefits. Our frog example can be viewed as a form of a collective memory. We suggest that collective memories that are important for a population's persistence are a category of distributed adaptation (e.g., Kaczensky et al., 2011); however, the more interesting among them, and those in which the distributed adaptations' perspective may be particularly useful, are cases in which different individuals carry different information and their sum is greater than its parts: those in which no single individual has the relevant information (Seeley et al., 2006). In particular, no individual has the "correct" or best information. The adaptive information arises from combining signals from multiple individuals.

This case is also not a case of niche construction. It is similar in so far that in niche construction multiple individuals contribute to the changes in the niche. Yet, in this case there are no persistent changes to the environment, on the one hand, and individuals make use of the distributed information, and integrate multiple pieces of information, on the other hand. These differences between distributed adaptation and related notions all lead to empirically testable predictions. For example, it is possible to empirically study whether different frogs have different phenotypes or behaviors (suggesting division of labor) and whether there are persistent changes to the environment that affect selection pressures or development (suggesting a niche construction explanation). Conversely, to test a distributed adaptation hypothesis it is possible to manipulate the information that is potentially aggregated, by experimentally producing croaking sounds and seeing how they affect the behavior of the frogs; manipulating the sound levels (e.g., by obstructing them); and so on; and seeing how these manipulations affect frogs' navigation. It is also possible to construct mathematical models and simulations to study the effects of such manipulations theoretically.

It might be tempting to consider only the croaking and moving in the direction of croaking sounds to be the adaptation. However, this perspective would miss the fact that these are adaptive only in relation to properties of the population (i.e., the number and distribution of frogs in the terrain), properties of the environment (i.e., the difficulty of navigation, terrain features that block sounds, etc.), and the relation between these factors. This relation concerns population level properties. That said, a key set of questions prompted by the notion of distributed adaptation involves the degree to which individuals rely on distributed information as opposed to individual traits and individual learning, and whether and how this is shaped evolutionarily. Consider a second thought experiment. Imagine a larva that transforms into a beetle. The imaginary beetles have the genetic capacity to produce several pigment colors; however, during transformation an irrevocable choice of one of them must be made. The color of the beetle affects the success of camouflage and hence survival. The best camouflage color depends on the type of predator that is prevalent at a given moment: birds, spiders, and lizards have different eyesight, and imagine that each can spot certain colors more easily than others. One possibility is for the larvae to choose a color randomly, in frequencies that are optimized evolutionarily. Another possible adaptation is to sense the colors of beetles in the vicinity and preferentially choose the color of the majority. If survival depends on having the right color, the relative prevalence of colors among adults that were not predated upon is a reliable indication of which color is currently preferable. As in the previous example, the key point is that the information about "best" color is not known to any individual nor genetically determined, but rather is spread in the population. As in the previous example, the ability to sense the color of conspecifics and use it to choose which color to adopt may evolve from an existing ability to detect colors in the environment.

The ability of a population to acquire and utilize information transcends the ability of a single individual in many well-known cases (Sosna et al., 2019). Consider vultures' foraging for carcasses to feed on: a single individual has a relatively low probability of finding food. However, by flying at a high altitude while keeping eye-contact with each other, groups of vultures jointly survey vast landscapes. When a vulture identifies a carcass it dives down, attracting others to dive in the same direction (Houston, 1974; Buckley, 1996; Jackson et al., 2008). This example illustrates that even over short periods of time distributed information may be crucial for survival [see also in this context the concept of emergent synergies discussed in Keenan and McShea (2021)].

One may wonder regarding the role of intentionality in DAs and about the relation between individual-level adaptation and DAs. Should a population-level adaptation be considered a DA only if individuals share their information with others in a way that seems intentional, as in a case of frogs' croaking, or vice versa? Should it be viewed as a DA only if it is also adaptive to the individual? For example, in the short term it is likely that a vulture could benefit from making its descent toward a carcass go unnoticed, if this were possible, thus decreasing the competition that it will experience while feeding (see also Rendell et al., 2010). Perhaps a "true" DA is only one in which adaptive value is gained at the population level and not—in the short term—by the individual? We suggest that these considerations are important, with crucial implications for understanding and predicting evolutionary dynamics of each studied scenario, but should be viewed as orthogonal to the question of which of these cases is considered a DA. We suggest that a DA perspective can yield insights regarding each of these scenarios. Interestingly, these considerations also influence whether a DA is likely to evolve in the first place and whether it is likely to persist: if sharing of information is not in the best interest of individuals, it is unlikely to emerge, unless avoidance of information-sharing has an even greater cost. Thus, for example, it may be that the frogs' calling, which attracts competing individuals, is maladaptive at the individual level in the short term but cannot be avoided because calling is also the frogs' means of attracting mates. Full treatment of these factors' roles in the evolution and maintenance of DAs is beyond the scope of the current study.

What distinguishes distributed adaptations from other forms of adaptation is that the population functions as a *distributed information store*. The information needed to produce the adaptive trait of an individual cannot be reduced to information found in a single genome or carried by a single individual's microbiome, memory, or morphological phenotype but instead consists of interactions between multiple individuals in the population and their ecological interaction with the environment.

This observation paves the path to a generalization of the concept: it is reasonable to consider the *population-environment complex,* and not only the population in itself, as often providing the necessary information for an individual's survival (Blanchet et al., 2010). This information may be embedded in the features of the population's spatial distribution, its behavior, or its interactions with elements in the environment. This dependency on the environment may be, for example, the availability of other individuals of the same species (as in our original example), presence of individuals of other species, or various other aspects of the biotic or abiotic environment; these can be structural, nutritional, chemical, etc. We refer to complex cases of distributed adaptation, in which properties of the population are significant and especially when the phenotypes of individuals depend on population-level features and their relations to the environment are critical for understanding the system, as *conformational adaptations.*

Cultural adaptations may be viewed as distributed adaptations. In simple cases it is possible for an individual to learn from a single other individual, while the population may embody additional information, such as the frequencies of different behaviors and their payoffs, which organisms may evolve to utilize. In more complex cases, an individual acquires the information it requires by combining information from multiple other individuals. Often, the acquisition has to occur in a specific order. The availability and salience of information may affect the developmental trajectory of individuals.

Distributed adaptations depend on the population or ecosystem level; however, the claim is not that they are the result of group selection or multi-level selection (Smith, 1976; Wilson, 1983, 1975; Dugatkin and Reeve, 1994; Kerr and Godfrey-Smith, 2002; Okasha, 2006; Traulsen et al., 2008; Eldakar and Wilson, 2011). The notion of a distributed adaptation refers to the location of the adaptive information, and not to how this distribution of information and reliance on distributed information came about evolutionarily or its inheritance (cf. Charbonneau, 2014). Put differently, when we talk about a distributed adaptation, we are not attributing fitness to the population or group, and the cases we discuss in this paper are primarily concerned with distributed information that affects the fitness of individuals. Moreover, a population may manifest a distributed adaptation without being a replicator/reproducer (Lloyd, 2016). Indeed, information may even be acquired from different species (Stensland et al., 2003; Sridhar et al., 2009; Farine et al., 2012; Gil et al., 2018), though in this paper we focus primarily on cases involving the dynamics of a single population.

3. RELATION TO OTHER PHENOMENA

A wide variety of species rely on social information, including fish, birds, and mammals (Danchin et al., 2004; Galef and Laland, 2005; Valone, 2007; Blanchet et al., 2010; Rieucau and Giraldeau, 2011). The social information is used in foraging, in avoiding predators, assessing habitats, and so on [for a review see (Gil et al., 2018)].

Several theoretical notions that have been studied previously are possibly special cases of distributed adaptation. Among them division of labor (Robinson, 1992; Beshers and Fewell, 2001), the skill pool effect (Giraldeau, 1984), strategy choice (Kendal et al., 2009; Rendell et al., 2010), distributed cognition (Hutchins, 1991; Cole and Engeström, 1993), and collective memory (Couzin et al., 2002; De Luca et al., 2014). However, the analyses of these often assume that the information is stored by specific individuals and that it can be transmitted from one individual to another individual, whether through social learning or inheritance. The general case of distributed adaptation considers situations in which the adaptive information cannot be reduced to anything that is possessed by any single individual, whether in their brains or genes. Thus, not all cases of social information involve distributed information in the sense we highlight. Moreover, not all cases of distributed information are cases of distributed adaptation, that is, distributed information is not necessarily adaptive. Finally, distributed adaptations need not involve explicit transmission of information between individuals and may result from their ecological activities, spatial conformation and so on (see **Figure 1**).

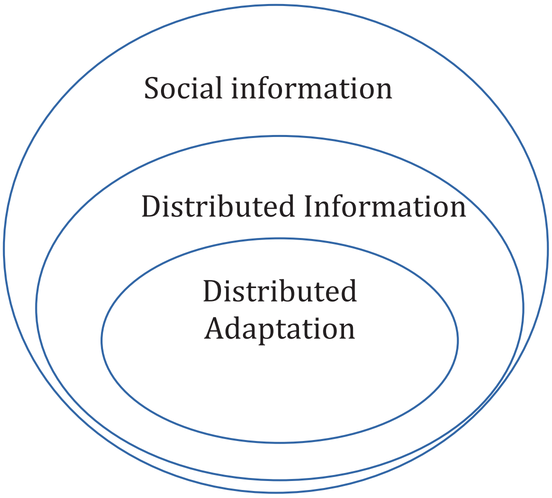

FIGURE 1 | The relations between the theoretical notions of social information, distributed information, and distributed adaptation. Social information does not always involve information that is distributed, in the sense of not being easily partitioned into information stored by specific individuals. Not all cases of distributed information in a population are cases of distributed adaptation. Note that the figure represents only distributed adaptations that involve explicit transmission of information between individuals (see text).

It is useful to consider a couple of these phenomena from a perspective of distributed adaptations. In conformist learning, individuals sample several models and pick what is most frequent to imitate (Henrich and Boyd, 1998; Aplin et al., 2017, 2015; Smaldino et al., 2018). Conformism depends on the prevalence of behaviors and the prevalence is not information possessed by any individual. Thus, it may be instructive in some cases to consider conformism as a specific case of distributed adaptation in which there are two pieces of information that play an adaptive role: the behavior and its prevalence. The information about prevalence is distributed and conformist learning is a sampling mechanism for (approximately) acquiring it. Making this distinction explicit may for example be productive in cases in which conformism interacts in interesting ways with population structure and ecological context.

Collective memory refers to cases in which the population as a whole remembers and is able to access information that exceeds the memory of individual members. A typical set of examples involves memories of older individuals, produced by longer experience or experience of different environmental conditions (Morales et al., 2010). It may be possible for the population to utilize such information, by imitating the older individuals, when an adverse condition reoccurs (e.g., Kaczensky et al., 2011). There are two questions that can help distinguish between cases of collective memory and distributed adaptations. (1) Can the information be acquired from a single individual (e.g., one older individual)? (2) Does the information persist in the population through chains of individuals, or does it involve interactions between multiple individuals or group level behaviors (e.g., flocking, collective grazing)? In practice, many cases of collective memory are to some extent also cases of a distributed adaptation. For example, consider a scenario in which the information about an adaptive destination of migration in a certain rare event is carried by individuals. However, to reach a decision to carry out such a migration, the group might need to make a quorum decision, requiring that several experienced individuals all support the unusual direction of migration (Conradt and Roper, 2005; Couzin et al., 2005; Harel et al., 2021).

Other phenomena that partially overlap with distributed adaptations are cases of local enhancement and conspecific cuing (Muller et al., 1997; Greene and Stamps, 2001; Doligez et al., 2003; Donahue, 2006; Rendell et al., 2011; Galef, 2013; Arbilly and Laland, 2014), that consider dynamics in which individuals are attracted to localities in which conspecifics occur or engage in certain behaviors (feeding or mating for example). These cases often overlap with cases of distributed adaptation—as in the frog example provided above, which can be described in these terms. We suggest that the notion of distributed adaptations is useful even when a phenomenon is well-described by the concepts of local enhancement or conspecific cueing: the latter are typically invoked to explain behavior, to provide an account of a behavioral mechanism. One might debate, for example, whether chickadees learn to open milk bottles *via* individual trial and error coupled with local enhancement, or whether true imitation is involved in the process (Fisher and Hinde, 1949; Sherry and Galef, 1984; Aplin et al., 2013). Local enhancement and conspecific cueing are rarely invoked to describe an evolutionary adaptation of a species to its environment. In other words, the different terms invoke consideration of implications on different time scales and in different contexts. Local enhancement is only rarely invoked when planning reintroduction programs of a species into the wild, for example, while the concept of distributed adaptation lends itself naturally to such contexts, providing a useful framework for the explicit treatment of this important facet of population success (Reed and Dobson, 1993; Dobson and Poole, 1998; Ahlering and Faaborg, 2006; Halpern et al., 2007; James et al., 2015). More speculatively, this discussion suggests that local enhancement can lead to population-level results that may be studied using the notion of distributed adaptation.

In a similar vein, distributed adaptations have much in common, and in some cases overlap, with phenomena that are described and studied in complex systems' research: self-organization, collective behavior, and emergent properties (Green, 1993; Lansing and Kremer, 1993; Parrish et al., 2002; Couzin and Krause, 2003; Goldstone and Gureckis, 2009; Lukeman et al., 2010). Here, too, we suggest that the different concepts implicitly invoke different perspectives: research in the field of complexity focuses on the explanation of observed behaviors of dynamical systems, such as patterns that emerge from the interaction of multiple individuals without explicit coordination. They are not readily translatable to discussion of a species' robustness to environmental change or to risk assessments of bottlenecked populations. They are also not primarily concerned with the evolutionary dynamics of interchange between individual level and distributed adaptation. Due to limitations of scope, a full analysis of the relation between distributed adaptations and these many related notions will await future research.

Finally, there are many instances in which achievement of adaptive goals relies on joint action by multiple individuals, possibly involving distributed information or communication for successful coordination. These include, for example, coordinated attack of trees by bark beetles (Chiu et al., 2018; Toffin et al., 2018), or pack hunting among wolves (Mech, 2007). Phenomena such as these are often studied under the heading of collective action. It is debatable whether all these cases should be considered to be DAs. We put off this discussion for future work but offer the following preliminary observations. The key feature of DA is that there is something interesting in the way that the population and its structure are the substrate for the adaptation of the species to its environment. Cases in which the number or density of organisms each doing their own thing explain the observed behavior are probably best viewed as degenerate cases of DA. Distributed, heterogeneous, behaviors are better signs of a DA. Two characteristics in particular may suggest that the DA perspective would be useful in a certain context. First, that there is good reason to consider the phenomenon to be an adaptation to specific life challenges or to have evolved in such a context. Second, that there are specific, non-trivial, individual traits or a population structure that are necessary for the collective result. Whether the DA perspective is useful also depends on the question of interest. For example, group hunting of colobus monkeys by chimpanzees (Boesch and Boesch, 1989) may be analyzed in the context of social roles, in which case the populational perspective of DA is perhaps less useful. It would be insightful, however, if the question of interest is the population's ability to recover from demographic bottlenecks, for example.

4. IMPLICATIONS OF THE NOTION OF DISTRIBUTED ADAPTATION

Many categories of adaptation that we propose to view as distributed adaptations are well-known. What, then, is the added value of introducing a new concept?

We propose that this view is useful in assessing an adaptation's evolutionary stability and in predicting evolutionary dynamics. As noted earlier, distributed adaptations may readily arise, founded upon small adjustments of existing sensory and behavioral abilities of the species. However, due to the utter reliance on the environment and the population, distributed adaptations are highly sensitive to changes, and may readily fail. They can be viewed as a special category of niche specialization; it has been suggested that high niche specialization is prone to leading to evolutionary dead-ends (Haldane, 1951; Kelley and Farrell, 1998; Nosil and Mooers, 2005; Vamosi et al., 2014). Similarly, distributed adaptations are prone to collapse due to environmental instability. On the other hand, in certain cases distributed adaptations may be more robust than other adaptations, thanks to the redundancy provided by the population. In addition, when the behavior of organisms in the population dynamically adjusts to a changing or uncertain environment, the behavior at the population level may act as a "sensing device" that individuals can make use of as illustrated by the examples in section 2.

It is helpful to consider the evolutionary interaction between distributed and non-distributed adaptations to the same challenge: distributed adaptations can be viewed as cases in which crucial aspects of a species' survival were "outsourced" to the environment, such as when a species loses innate knowledge about utilization of a food resource and comes to rely on social learning of the information. The opposite also occurs, of course: the Baldwin effect (Baldwin, 1896; Weber and Depew, 2003; Crispo, 2007; Scheiner, 2014), or other cases of genetic assimilation, are in some cases distributed adaptations that are being replaced by individual-level adaptive information (Simpson, 1953; Sznajder et al., 2012). These evolutionary dynamics may interact with long-term selection processes and with species' demographic history to determine species' evolutionary trajectories and provide insight, for example, on the conditions that may foster or inhibit the emergence of cultural adaptation, or the evolution of learning and teaching abilities.

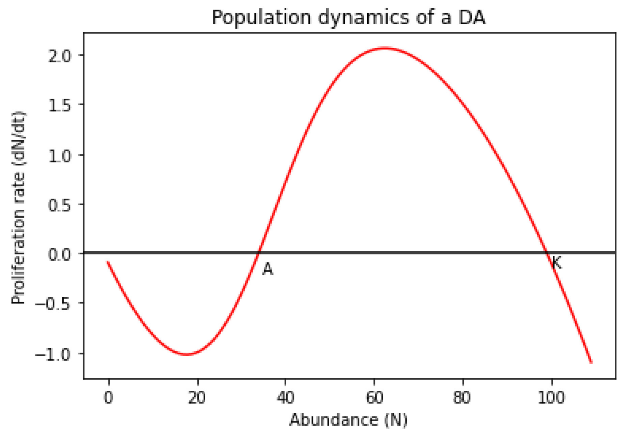

FIGURE 2 | An Allee effect produced because of distributed adaptation. When population size is smaller than *A*, not enough individuals express T_0 and the fitness is below 1. When the population size is greater than *A* the distributed information is expressed by the population and the reproductive success of individuals increases asymptotically to the maximal reproductive success, in check by competition for resources.

To illustrate possible implications of distributed adaptations, we will make use of a simple model. Consider a case in which the phenotype of individual i, p_i, depends on the number of individuals in the population that have a desired phenotype T_0. The idea is that T_0 is the phenotype that other individuals express allowing the focal individual to acquire the distributed information. In the example of the frogs navigating toward croaking conspecifics, T_0 is the croaking when in the ponds while p_i is effective navigation to ponds. The phenotype p_i is suboptimal and the population declines unless the population size is larger than A, in which case enough individuals express the required phenotype T_0. The phenotype becomes increasingly better, up to a point in which additional individuals are not required to "carry" the distributed adaptation. In other words, the population needs at least A individuals with the phenotype T_0. We assume that the average phenotype and fitness depends on the number of individuals with the required phenotype, so that on average the number of offspring is below 1 until $N \geq A$ and increases asymptotically to the maximal reproductive success when $N > A$.

Implication 1: Allee Effects and Distributed Adaptation

The *Allee effect* is used to describe cases in which the mean individual fitness increases with population size or density (Stephens and Sutherland, 1999; Stephens et al., 1999; Deredec and Courchamp, 2007). Different mechanisms have been proposed to drive Allee effects, but the causality is often unclear. Distributed adaption may explain Allee effects in some cases: in distributed adaptation, the ability of the population to "store" the adaptive information depends on the size of the population, since the information is stored in a distributed manner across it.

The toy model above illustrates a strong Allee effect driven by distributed adaptation. This is the simplest scenario, used for demonstration, and shows that interesting dynamics may emerge when adaptive information is distributed. The resulting population growth curve, under this model of a simple distributed adaptation, matches that of a typical Allee Effect (**Figure 2**).

Implication 2: Population Bottlenecks

The consequences of a population bottleneck depend on whether the new population size N' is smaller or larger than the minimal required number A of individuals needed for the distributed adaption. Some environmental changes that lead to a population bottleneck may involve a new, smaller A' (e.g., when the environment becomes less complex), while in general a population bottleneck does not change the required number of individuals, A. For illustration, consider two scenarios.

Simple Population Bottleneck

Here the population size is decreased, but the environment is otherwise unchanged, for example: $N' = N/2$, while the carrying capacity k and A remain fixed.

Population Bottleneck With Less Demanding Environment

In this scenario, the population bottleneck co-occurs with moving to an environment that is manageable with less outsourced information, and hence a smaller A. For example, a smaller niche, but with more resources per unit area, will have a denser population, thus increasing the probability of interactions between individuals, which reduces the number of individuals with the required phenotype that are needed to make acquisition from others robust. A second case in which A decreases is one in which the new environment is simpler in the sense that there is less information that needs to be stored by the population (e.g., more ponds, which are hence easier for the frogs to locate). In this kind of population bottleneck, for example, $N' = N/2, A' = A/2$, while k is unchanged.

If $N' > k'$ the population will collapse to k'. However, if $N' < k'$ the fate of the population depends on the relation between N' and A': if $N' > A'$ the population will increase to k' while if $N' < A'$ the population will decrease to 0. The key to the dynamics is whether A decreases in line with the decrease in N. The scenarios above suggest two cases in which this may happen: when the new environment is simpler or when increased population density makes the distributed information storage more efficient (thus requiring less individuals) than in the original environment. Both cases depend on the specific nature of the information outsourced and the properties of the organism that affect acquisition.

Implication 3: Population Reintroduction

This analysis shows why a small population (e.g., a species introduced to nature) may benefit from an artificial environment that is enriched, or simpler (thus, lower A), until the population increases enough in size to be introduced to an environment which requires a larger minimal population. However, that may not be enough: if the population needs to organize to capture/represent the adaptive distributed information, it may be necessary to support the population for an interim period, until this is achieved. Note that these two things would have to follow each other: a population that is too small will not be able to capture the required information even if supported exogenously.

Implication 4: Invasive Species

Distributed adaptation also suggests a possible explanation of a gradual adaptation to the environment in invasive species. Many species are characterized by an invasion time lag: a long period of limited success between the time point of establishment and until they become invasive, spreading rapidly and driving significant changes to the ecosystem (Crooks, 2005; Coutts et al., 2018). There are different explanations to what causes this waiting period and what ends it; the notion of distributed adaptations adds the possibility that it takes time for the distributed information to emerge or build up, possibly requiring the invasive population to be large enough. This is not unlike niche construction.

Implication 5: Evolutionary Dynamics

The model discussed so far illustrated the effects of a DA on the success of a population in various scenarios. We now turn to the evolutionary interaction between distributed and non-distributed adaptations to the same challenge. We can think of an organism evolving to use external adaptive information from the population or resulting from the activities of the population, instead of relying on genetic information, as externalizing the information. Conversely, moving to rely on genetic information instead of distributed information may be viewed as internalizing the adaptive information. Internalizing and externalizing both have potential costs and benefits. Relying on a DA may lead to fragility and is sensitive to changes in population size and bottlenecks, as illustrated above. Its benefits include the advantages of redundancy and fault-tolerance more broadly, since the organism does not rely on one copy of the information (i.e., its own genome) but rather on multiple individuals in the population, which would typically mean not relying on any specific individual. Externalizing may in some cases allow the organism to reduce the size of its genome or to save the cost of development and maintenance of morphological or neural substrates that would have otherwise been necessary. Thus, for example, if the non-DA solution requires complicated behaviors or trial-and-error learning, the DA may allow reduction of the size and complexity of the nervous system. In other cases, the non-DA solution may necessitate a specialized sensing apparatus and neural substrate that supports its operation, whose maintenance can be spared if the DA relies on other substrates that must be maintained anyway (the frog and the nymph examples illustrate how this could work). Two important potential benefits of DAs are that they reduce the cost of exploration for the individual, since exploration costs are divided across the population, and that DA may change more quickly in response to environmental changes, in comparison to genetic changes (i.e., mutation and selection) or individual learning.

The costs and benefits of internalization are the mirror image of this. Relying on genetically endowed abilities makes the individual less sensitive to what others are doing, to evolutionary cheating, to conflicts of interest, and so on. Internalization is also a way to decrease the time and resources needed to collect information or cues from the population. This may have an advantage for the individual as illustrated in the discussion of reintroduction and of invasive species above. It may also be advantageous at the population level, increasing in some cases its chances of responding to changes and recovering from bottlenecks.

One situation that may lead to externalization is relaxed selection (Lahti et al., 2009; Hunt et al., 2011; Schrader et al., 2021). Consider as an example the frog scenario and assume that there are two genes: gene A contributing to the ability of the frog to navigate the terrain in search of ponds (think of this as the non-DA solution) and gene B that allows the frogs to go in the direction of croaking (this gene may for example be involved in mating). If the population is large enough such that croaking is a reliable signal for the location of ponds, differences in the navigation abilities (i.e., in gene A) would not significantly affect the individual frogs' fitness. This relaxed selection on A may lead the gene to accumulate mutations and potentially cease being functional. Thus, the frogs would end up relying exclusively on going in the direction of croaking sounds to find ponds. The individual level adaptation represented by gene A is thus replaced by a DA.

The opposite may also happen. For example, if at some point during the evolutionary scenario just described the population dwindles, making the croaking sounds less useful for navigation, selection in favor of better navigation skills (gene A) would become stronger and an individual-level adaptation may spread and fix. Assume further that the terrain is stable enough that a navigation strategy is clearly superior to others and that relying on sounds may lead to mistakes, perhaps because a related species makes similar croaking sounds but inhabits drier areas. In such a case there would be selection against relying on the external information.

A variety of properties of the population may affect phenotypes that are influenced by outsourced information. In the examples above we illustrated the role of population size. Roughly put, if relevant information is somehow encoded in the composition, structure, or dynamics of the population, the size of the population may be too small to carry the amount of information needed for the adaptation, while a large population may possibly encode information more robustly than small ones (e.g., by sheer redundancy). Demographic collapse may lead distributed information to be lost, which cannot simply be recreated when population size increases. The following may all be affected by population size: the stability of the traits over the lifetime of individuals; the heritability of traits; the quality of the trait (e.g., larger population produce better distributed adaptation); whether a population maintains a stable population level distributed adaptation, and in consequence maintains a viable population size. We focus here on population size *per se* for clarity, however, additional population-level features may have related and partially overlapping effects. These include population structure and networks of interaction, on a broad range of timescales, from day-to-day interactions to rare events of inter-population migration that occur once in a generation, for example. Similarly, life history and demographic structure may influence distributed adaptations, and distributed adaptations

TABLE 1 | Factors affecting the evolution of distributed adaptations.

	Benefits	Costs	Process
External(izing)	Redundancy Fault tolerance Sensitive to env. (in parallel) Reduce genome/CNS Lower cost of exploration (∼recessivity) Faster change (e.g., culture)	Fragility Sensitive to pop. size e.g., population bottlenecks	Relaxed selection Selection (?)
Internal(izing)	Less prone to cheating “faster” acquisition	Size of genome/CNS Developmental costs and constraints	Selection Baldwin Effect Genetic Assimilation

Externalizing refers to increased reliance on distributed information; internalization for decreased reliance on distributed information.
See text for discussion.

may even influence selection on these traits in certain conditions. Thus, for example, the robustness of a distributed adaptation may be strongly influenced if individuals at different ages contribute to the dynamics related to it differently.

Whether an equilibrium between relying on individual level or distributed adaptation exists and in what contexts natural selection can fine-tune the distribution of an adaptation are currently open questions. **Table 1** summarizes the factors discussed in this section.

5. CONCLUDING REMARKS

The notion of distributed adaptations introduced in this paper complements and generalizes other evolutionary notions, including social information, niche construction and developmental niche construction, distributed skills and the skill pool effect, and work on strategy choice and conformism. It directs our theoretical focus to the role of information that is distributed in the population and its ecological conformation. Finally, we suggest that distributed adaptations have implications for conservation biology and in particular for re-introduction efforts.

We illustrated the notion of distributed adaptation with two simplified but realistic examples: frogs navigating an arid terrain and nymphs color choice. The first adaptation makes use of the spatial distribution of the population and the second makes use of the population as a way to sense the frequency of predators. Crucially, in both cases no single individual has the adaptive information, and cannot store it, transmit it to others etc. Yet in both these cases the evolutionary beneficiary is the individual and it is adaptive for individuals to make use of the distributed information.

By taking seriously the consequences of evolutionary relevant information that transcends an individual brain or genome, and that is encoded in interaction networks and environmental conformation, a coevolutionary approach to distributed adaptations offers explanatory traction regarding the effects of population size and dynamics, environmental stability, and properties of information acquisition, in particular its reliability. This suggests general answers as to why and when information may be distributed as well as concerning the natural history of distributed adaptations. Our informal examples suggested an important role for sensory pre-adaptations that allow individuals to profit from being sensitive to the conditions of the population. This perspective may also help identify the factors that explain the variety of distributed information storage phenomena.

More generally, a formal framework of distributed adaptations connects population level and individual properties to derive specific predictions about the cost/benefits that affect internalization and externalization of information, and the resulting evolutionary processes (see **Table 1**). It sheds light on dynamics that exist in various evolutionary models of social and cultural evolution, but that so far have not been addressed under a unified framework.

An important theoretical insight stemming from the notion of distributed adaptations is that evolutionary relevant information that is distributed in the population can spontaneously emerge once individuals can utilize such information. This need not require selection between groups. We illustrated this by showing that when information is available as a result of the typical activities of members of the population it can be evolutionarily beneficial for individuals to make use of them in favor of more costly, individual-level adaptations. Distributed adaptations may lead to coevolutionary dynamics between traits that rely on outsourced information and traits that improve the ability of a population to serve as a distributed information store. One significant example in human evolution may be coevolutionary dynamics between emotional control, which facilitates social learning, and individual learning: Better emotional control, within well-functioning groups, will reduce the selection pressure on individual problem-solving, thereby in return increasing the selection pressure for emotional control.

The analysis of distributed adaptation suggests that in addition to the types of evolutionary relevant individual information that have been discussed, specifically the genetic, epigenetic, behavioral and symbol information systems (Jablonka and Lamb, 2014), we should pay attention to ways population-level information systems evolve. This study may benefit from ideas developed by computer scientists studying distributed systems (Lynch, 1996). The notion of a memory hierarchy has been developed to classify computer storage systems according to their characteristics (primarily, response time), with the design goal of storing data using the lowest-cost components, that still allow the system to satisfy requirements (Hennessy and Patterson, 2011). Mutatis mutandis, similar things may happen in evolution: when considering costs, we should take into account the time and selection required to achieve an individual adaptation compared to the alternative distributed adaptation, the robustness or fragility of the individual versus the distributed adaptation, and so on. Genome size and genomic architecture may in some cases be constraints, for example, increasing the likelihood of the evolution or stability of one type of adaptation or the other, e.g., by facilitating the acquisition of new functions by existing mechanisms or hindering it. Similarly, selection pressures on the size or complexity of the brain may favor either the individual or the distributed route (**Table 1**).

The notion of DA highlights that information can be distributed in various ways (in individuals, in the entire population, in different population structures). The costs and benefits change, and the question is how and whether this distribution is subject to evolutionary change, in particular tuning by natural selection. e.g., tradeoffs between genome size or central nervous system complexity and population size.

Moreover, the notion of DA will hopefully inspire more biologists to consider theoretical constraints on what distributed systems with failure can do. For example, a classic result shows that a system may not reach consensus if more than a third of the agents give conflicting information to different agents (see Lynch, 1996). While a biological population should not typically be considered a "system" in the sense used in computer science, distribution of information and the need to integrate distributed information raises similar issues and the notion of DA may bring these issues to the attention of biologists.

The notion of niche construction was introduced to highlight that the fit between an organism and the environment stems not only from information flowing through selection "into" the genome of the organism but also from information that flows from the organism to the environment (Odling-Smee et al., 2003). The notion of developmental niche construction was meant to capture the fact that the constructed niche affects not only the fitness of individuals but also their development (Stotz, 2017). The notions suggested here extend these discussions. Distributed adaptations are cases in which the developmental trajectory of individuals is affected by the exhibited phenotypes in the population, which may change over time, by the distribution of phenotypes, as well by as the conformation of the population in the environment. However, as noted earlier, in the cases we highlighted there are no persistent changes to the environment, as found in niche construction, that indirectly affect the selection pressure affecting individuals in the population. In contrast, in the cases we highlighted individuals make direct use of the distributed information and integrate multiple pieces of information.

Major Transitions in Evolution were characterized by Maynard Smith and Szathmáry as transitions creating a new level of individuality and new kinds of information systems, that is, new ways of storing, transmitting, and interpreting information (Jablonka and Lamb, 2006; Robin et al., 2021). Our paper focuses on the latter. While it was suggested that these two dimensions should be treated separately, one assumption in particular remained prevalent in most subsequent work. Namely, the view that information is at the level of individuals. In other words, the information that is primarily tracked in such studies, whether theoretical or empirical, is information that can be stored and transmitted by and between individuals. This assumption may explain why it is tempting in studies of evolutionary transitions in individuality to wonder if new kinds of individuals are coupled with new kinds of information. Be that as it may, our discussion emphasizes and characterizes a supplementary perspective: cases in which adaptive information strictly transcends the level of individuals. It may even be thought of as a level of individuality, though one that is distinct from the biological individuality of individual organisms.

Two aspects of our characterization of DA may have implications for thinking about evolutionary transitions. DAs are cases in which (1) the adaptive information is distributed, and hence strictly the property of a population and (2) the information may arise from the ecological conformation of the population. We discussed the evolutionary implications of DA in section 4. As regards evolutionary transitions in particular, two possibilities are worth considering. First, DAs may contribute to the irreversibility of transitions. We noted that relying on a DA may affect selection pressures, causing relaxed selection on traits that allow the individual to be self-sufficient, possibly leading to the external information becoming increasingly necessary and the reliance on it mandatory. Change along this continuum may make the possibility of reverting to prior organization less likely. Second, since distributed information may arise spontaneously and may provide ecological information that may be otherwise hard to acquire, they may be an enabling factor that facilitates a transition or in some cases a necessary step, that is, they may be part of what Robin et al. (2021) call a Facilitating Evolutionary Transition.

An early example of a transition from distributed to non-distributed information may be what Carl Woese referred to as the Darwinian Threshold in the evolution of the cell (Woese, 2002). In this scenario, proto-cells relied heavily on horizontal gene transfer, but as the complexity of cells increased this became too unreliable, and vertical transmission became dominant. Woese referred to this as a phase transition. While this scenario has many unique features, not found in other cases, the evolutionary dynamics suggested by Woese are not entirely dissimilar from what we describe.

Two other transitions are worth mentioning here. The first, not included in Maynard Smith and Szathmáry's original list, is the evolution of a nervous system (Jablonka and Lamb, 2006). The existence of a nervous system and sensory perception greatly increased the possibility of relying on distributed information, whether intentionally shared or not. The second is the emergence of symbolic language. Language obviously radically increases the opportunities to learn from others and is fundamental for cumulative culture in humans, aspects of which are arguably DAs. While it is beyond our scope to discuss language and its evolution (but see Lamm, 2014; Kolodny and Edelman, 2018), it is worth noting that recent work has identified relations between group size and properties of language, as would be expected from our discussion of DA (Lupyan and Dale, 2010; Atkinson et al., 2018; Raviv et al., 2019). An intriguing aspect of language as an information system is that it transcends a particular group and several groups may share languages while individuals may be multi-lingual (Evans, 2017). By acquiring a language one does not only increase the opportunities to communicate but also acquires information that is implicit in the structure of the language itself, such as categories of objects or events. This is arguably an additional way in which information distribution occurs in humans.

Robin et al. (2021) characterize five levels of information in the context of evolutionary transitions. This characterization allows to differentiate several kinds of DA. The frog example which we discussed throughout the paper belongs to what they call Level III. This level refers to learned information, collected during the lifetime of individuals. Other cases of DA would more naturally fall under Level IV, which refers to inscribed or iconic information. The information at this level is transmitted using somewhat persistent physical marks, as in scent marks in wolves or pheromone trails in ants. This level also includes instructional information, of potentially boundless capacity, that is primarily found in humans. These cases are forms of distributed information or can be used to create distributed information, in the sense developed here. A key aspect of the Level IV phenomena as DA is that the objects that carry the information may be themselves studied as a population of entities, whose dynamics are coupled with those of the population of organisms that create, use, and possibly destroy them.

To conclude, distributed adaptations may be a stage in transitions in individuality, possibly because of the fragility of the distributed adaptation vis-à-vis the environment (Szathmary and Smith, 1995; Smith and Szathmary, 1997; Michod, 2000; Jablonka and Lamb, 2006; Calcott and Sterelny, 2011; Szathmáry, 2015). A distributed adaptation means that multiple individuals gain shared benefits from maintaining the distributed information. Given appropriate conditions, this may lead to the evolution of stronger ties and mechanisms to decrease the fragility of the distributed adaption, possibly leading to a transition.

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

Both authors contributed to the study equally, conceptualizing the paradigm and writing the manuscript together.

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Design for an Individual: Connectionist Approaches to the Evolutionary Transitions in Individuality

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The truly surprising thing about evolution is not how it makes individuals better adapted to their environment, but how it makes individuals. All individuals are made of parts that used to be individuals themselves, e.g., multicellular organisms from unicellular organisms. In such evolutionary transitions in individuality, the organised structure of relationships between component parts causes them to work together, creating a new organismic entity and a new evolutionary unit on which selection can act. However, the principles of these transitions remain poorly understood. In particular, the process of transition must be explained by "bottom-up" selection, i.e., on the existing lower-level evolutionary units, without presupposing the higher-level evolutionary unit we are trying to explain. In this hypothesis and theory manuscript we address the conditions for evolutionary transitions in individuality by exploiting adaptive principles already known in learning systems. *Connectionist* learning models, well-studied in neural networks, demonstrate how networks of organised functional relationships between components, sufficient to exhibit information integration and collective action, can be produced via fully-distributed and unsupervised learning principles, i.e., without centralised control or an external teacher. Evolutionary connectionism translates these distributed learning principles into the domain of natural selection, and suggests how relationships among evolutionary units could become adaptively organised by selection from below without presupposing genetic relatedness or selection on collectives. In this manuscript, we address how connectionist models with a particular interaction structure might explain transitions in individuality. We explore the relationship between the interaction structures necessary for (a) evolutionary individuality (where the evolution of the whole is a non-decomposable function of the evolution of the parts), (b) organismic individuality (where the development and behaviour of the whole is a non-decomposable function of the behaviour of component parts) and (c) non-linearly separable functions, familiar in connectionist models (where the output of the network is a non-decomposable function of the inputs). Specifically, we hypothesise that the conditions necessary to evolve a new level of individuality are described by the conditions necessary to learn non-decomposable functions of this type (or deep model induction) familiar in connectionist models of cognition and learning.

Keywords: evolution, deep learning, evolutionary connectionism, basal cognition, development, natural selection, adaptation, multi-level selection

INTRODUCTION: EVOLUTIONARY TRANSITIONS IN INDIVIDUALITY

All complex individuals are made of parts that used to be individuals themselves (e.g., the transition from single-celled life to multicellular organisms). Such *evolutionary transitions in individuality* have occurred at many levels of biological organisation, and have been fundamental to the origin of biological complexity, but how they occurred is not well understood (Maynard Smith and Szathmary, 1997; Michod, 2000; Okasha, 2006; Godfrey-Smith, 2009; Szathmary, 2015; West et al., 2015). Before a transition, adaptations under natural selection support component entities in acting to maintain their individual survival and reproduction. But after a transition, natural selection supports components in acting to serve the development, survival and reproduction of an individual at higher level of organisation (e.g., the multicellular organism), even when it conflicts with or suppresses the survival and reproduction of these component parts (e.g., somatic cells) (Maynard Smith and Szathmary, 1997; Godfrey-Smith, 2009).

How do they come to work together in this way? The form and function of the many different parts within an individual, and their working together as a coordinated whole, is consistent with natural selection acting at the higher level. When the higher-level individual is established as an evolutionary unit, i.e., after a transition, this can even explain self-sacrifice at the level of component parts – as they are no longer effective evolutionary units as individuals. But this presupposes the higher-level individual as an evolutionary unit and does not explain the process of the transition. The evolutionary changes involved in the creation and maintenance of a new level of individuality are complex and can involve many evolutionary steps in multiple dimensions including population structure, functional interdependence and reproductive specialisation (Godfrey-Smith, 2009). For example, these may include: a new kind of compartmentalisation (e.g., cell membrane) that limits the distribution of public goods or provides physical protection that binds selective fates together; new social relationships that create irreversible fitness dependencies between ecological partners (e.g., from ecological "trade" to division of labour); the synchronisation and centralisation of reproductive machinery (e.g., as in the origin of chromosomes and the eukaryote cell); changes to physical population structure that implement genetic assortment (e.g., a reproductive bottleneck in the origin of multicellular animals) and/or reproductive specialisation (with early-determination and sequestration of a germ line) (Margulis and Fester, 1991; Maynard Smith and Szathmary, 1997; Michod, 2000; Okasha, 2006; Godfrey-Smith, 2009; Buss, 2014; Szathmary, 2015; West et al., 2015). Such changes cannot be explained as adaptations of the higher-level unit because the higher-level unit does not exist until after (some sufficient subset of) these adaptations have taken place. Rather they must be a result of selection on the extant lower-level units changing their functional relationships with one another. That is, evolutionary transitions in individuality must be understood as evolved or coevolved changes to relationships between existing evolutionary units – not as some kind of instantaneous jump in the unit of selection (followed by the evolutionary complexification of internal relationships and mechanisms) (Black et al., 2020; Veit, 2021).

This presents an evolutionary puzzle because, whilst the collective benefit of adaptations at the higher level may be significant in the long term, natural selection is famously short-sighted and self-interested. That is, characteristics that decrease immediate benefit, or differentially benefit others, do not increase in frequency. Selection at the higher level of organisation necessary to overcome this is not effective until *after* the new level of individuality is constructed, and selection at the lower level will not favour any changes that decrease short-term individual fitness (Veit, 2021). Assuming the new evolutionary unit did not spring into existence "all at once," with the necessary organised relationships already in place, the multiple changes involved in its creation must have been driven "bottom-up" by the selective interests of the extant, lower-level units – even though these same units are consequently caused to give-up their self-interest in the process. A key question in the transitions is thus:

How do multiple short-sighted, self-interested entities organise their relationships with one another to create a new level of individuality, meaning that they are caused by these relationships to act in a manner that is consistent with long-term collective interest?

To answer this, a theory of ETIs needs to describe (a) what kind of functional relationships between components are needed to make a new individual, and how they need to be organised; and (b) how the organisation of these relationships arises "bottom-up," i.e., without presupposing the higher-level individual we are trying to explain.

Existing evolutionary theory struggles with these questions. Specifically, a conventional evolutionary framework cannot explain adaptations in systems that are not evolutionary units. After the transition, when the higher-level individual is established as an evolutionary unit, selection at this higher level can explain complex relationships and even altruistic behaviours among the component parts. But before the transition, we cannot invoke natural selection to explain the adaptation of such system-level relationships or behaviours. Thus, if these are adaptations required to create the new level of individuality, how can selection explain them? Questions about how new units are created, or transition from one level of organisation to another, cannot be addressed within a framework that presupposes the unit it is trying to explain. As Veit puts it, the problem is one of circular reasoning: "how to explain the origins of Darwinian properties without already invoking their presence at the level they emerge?" (Veit, 2021). So the process of ETIs under bottom-up selection creates a chicken-and-egg problem for conventional thinking; Which came first, the higher-level unit of selection required for complex adaptations, or the complex adaptations required to create the higher-level unit of selection? (Griesemer, 2005; Clarke, 2016).

In this manuscript, we outline the existing theoretical frameworks and hypotheses regarding the ETIs, and discuss their limitations – in particular, the problem of creating fitness differences at the collective level that are not just a by-product of fitness differences among particles, and how to explain the selective mechanisms by which the structures necessary to produce this transition can evolve bottom-up.

We then introduce some new experimental findings in developmental biology – namely, "basal cognition" and the separation of organismic individuality from genetics (Manicka and Levin, 2019a; Lyon et al., 2021a) and new perspectives on evolutionary processes, namely "evolutionary connectionism" (Watson et al., 2016), which deepens and expands the formal links between evolution and learning. The link between evolution and simple types of learning has often been noted (Skinner, 1981; Watson and Szathmary, 2016) but is sometimes interpreted in an uninteresting way; as if to say *Some types of learning are no more clever than random variation and selection*. But the formal equivalence between evolution and learning (Frank, 2009; Harper, 2009; Shalizi, 2009; Valiant, 2013; Chastain et al., 2014) also has a much more interesting implication, namely: *Evolution is more intelligent than we realised* (Watson and Szathmary, 2016). Evolutionary connectionism addresses the two questions above by utilising (a) the principles of distributed cognition, familiar in neural network models, to explain how the relationships between evolutionary units can produce something that is "more than the sum of the parts" in a formal sense, and b) the principles of distributed learning to address how evolving relationships can be organised bottom-up, without presupposing system-level feedback. This provides new ways of thinking about these questions, leading to a new hypothesis for what ETIs are and how the ETIs occur.

The core of the idea is that ETIs are the evolutionary equivalent of deep learning (LeCun et al., 2015) (i.e., multi-level model induction), familiar in connectionist models of cognition and learning (Watson et al., 2016; Watson and Szathmary, 2016; Czégel et al., 2018, 2019; Vanchurin et al., 2021). We hypothesise that this is not merely a descriptive analogy, but a functional equivalence (Watson and Szathmary, 2016) that describes the types of relationships required to support a new level of individuality and the selective conditions required for these relationships to arise bottom-up. Specifically, we hypothesise that (i) the type and organisation of functional relationships between components required for a new level of individuality are those which encode a specific but basic type of non-decomposable computational function (i.e., non-linearly separable functions), (ii) these relationships are enacted by the mechanisms of information integration and collective action ("basal cognition") observed in the developmental processes of organismic individuality, and (iii) the conditions necessary for natural selection to produce these organisations are described by the conditions for deep model induction.

EXISTING APPROACHES TO THE PROBLEM OF THE EVOLUTIONARY TRANSITIONS IN INDIVIDUALITY

The evolutionary transitions in individuality, ETIs, have been some of the most important innovations in the history of biological complexity (Maynard Smith and Szathmary, 1997; Michod, 2000; Godfrey-Smith, 2009; West et al., 2015). These include the transition from individual autocatalytic molecules to the first protocells, individual self-replicating genes to chromosomes, from simple bacterial cells to eukaryote cells containing multiple organelles, and from unicellular life to multicellular organisms (Maynard Smith and Szathmary, 1997; Michod, 2000). Each transition is characterised by the "de-Darwinisation" of units at the existing level of organisation and the "Darwinisation" of collectives at a higher-level of organisation (Godfrey-Smith, 2009). That is, at the lower level, each component part loses its ability to replicate independently – the most fundamental property of a Darwinian unit – and after the transition, can replicate only as part of a larger whole (Maynard Smith and Szathmary, 1997). Conversely, before the transition, reproduction does not occur at the collective level; and after the transition the collective exhibits heritable variation in fitness that belongs properly to this new level of organisation (Maynard Smith and Szathmary, 1997; Okasha, 2006).

Whereas conventional evolutionary theory takes individuality for granted, and assumes the unit of selection is fixed, it is now recognised that Darwinian individuality is a matter of degree in many dimensions (e.g., degree of genetic homogeneity, degree of functional integration, degree of reproductive specialisation) (Godfrey-Smith, 2009). The research programme of the ETIs seeks to understand the processes, mechanisms and drivers that cause evolutionary processes to move through this space of possibilities (Okasha, 2006; Godfrey-Smith, 2009).

Social Evolution Theory and Kin Selection

Social evolution theory, a general approach to explain social behaviour, notes that it is evolutionarily rational to cooperate with someone that makes more copies of you (or your genes). Thus, in the case that interactors are genetically related or homogeneous, as they can be in the case of the cells within a multicellular organism, for example, this can explain the altruism of the somatic cells (West et al., 2015; Birch, 2017).

The inclusive fitness perspective on ETIs, derived from this kind of social evolution theory, also offers a viewpoint that side-steps the whole problem. The question, as we posed it, asked why short-sighted self-interested individuals would act in a manner that opposes their individual interest to serve the interests of the whole. But an inclusive fitness perspective suggests this is wrong-headed because they were never different individuals in the first place – they were always of one genotype, and the multicellular organism is just a phenotype of this singular evolutionary unit. Problem solved?

For some purposes, it might be appropriate to view ETIs as an extreme point on the same continuum as other social behaviours. But genetic relatedness, kin selection or inclusive fitness do not explain all ETIs or even key examples such as multicellularity with homogeneous genetics.

First, acting with unity of purpose in multicellular organisms does not require genetic homogeneity (Grossberg, 1978; Levin, 2019, 2021b; Levin et al., 2019; Bechtel and Bich, 2021). Second, other transitions in individuality involve components that are genetically unrelated, for example, the transition from self-replicating molecules to chromosomes, and the transition from bacterial cells to eukaryote cells with multiple organelles (Maynard Smith and Szathmary, 1997). Third, and perhaps most important, social evolution theory only explains the cooperation that is expected *given* a certain interaction structure (i.e., determining whether those that interact are related). It does not explain *changes* in interaction structures that are necessary to increase or decrease genetic assortment, let alone to reach such extremes. Moreover, the genetic definition of individuality fails to address all the questions that are really interesting about individuality – not least how individuality changes from one level of organisation to another. By asserting that, both before and after the transition, the only relevant individual was the gene, this approach fails to address the meaning of the individual at all. Of course, it is common that the cells of multicellular organisms, especially animals, are for the most part genetically homogeneous. And given that they are, this can explain the apparently altruistic behaviours of soma. But this does not explain how this situation evolved, nor other instances of individuality that are not genetically homogeneous.

Evolved Change in Interaction Structure: Ecological Scaffolding and Social Niche Construction

One recent approach to explain how new interaction structures might evolve is ecological scaffolding (Black et al., 2020; Veit, 2021). That is, extrinsic ecological conditions, that are not in themselves adaptations and do not require selective explanation, create conditions where individuals live in a grouped or meta-population structure, e.g., microbial mats aggregated around water reed stems (Veit, 2021). The differential survival and reproduction of such sub-populations, e.g., in recolonising vacant locations, affords the possibility of higher-level selection (Wilson, 1975; Wade, 2016). Thus far in this account, nothing has evolved to support or maintain these structures; It is simply an assumption of fortuitous extrinsic conditions that alter population structure to create these different selective pressures. But from there it becomes more interesting. Given these conditions, individual selection at the lower level supports the evolution of characters that access synergistic fitness interactions, changing the relationships among the particles, and given that synergistic fitness interactions among particles have evolved, it is subsequently advantageous for particles to evolve traits that actively support this grouped population structure. Now the original extrinsic ecological conditions might change or cease, but the population structure necessary to support higher-level selection is nonetheless maintained, supported by the adaptations of the particles. That is, the ecological scaffolding becomes redundant, and is replaced by endogenous effects of characters produced by selection at the particle level. This ecological scaffolding thus provides a way to overcome the chicken-and-egg problem of the ETIs (by temporarily assuming the presence of a "chicken"). It does, however, depend on the initial assumption of extrinsic ecological conditions that happen to support higher-level selection in the first place. Moreover, if population structure changes evolutionary outcomes for individuals, and individuals have the ability to alter population structure, we must consider the possibility that rather than adapting to support the new level of selection they act to oppose or disrupt it, e.g., by evolving dispersal behaviours rather than aggregation behaviours.

These works and others in this area point to the need to explain how evolution modifies the parameters of its own operation when these parameters exhibit heritable variation (Powers and Watson, 2011; Ryan et al., 2016; Watson and Szathmary, 2016; Watson and Thies, 2019), i.e., to endogenise the explanation of its own parameter values (Bourrat, 2021b; Okasha, 2021). For example, with or without scaffolding, suppose that organisms have heritable variation in traits that modify their interaction structure with others, such as compartmentalisation or group size, reproductive synchronisation, or reproductive specialisation. These traits can modify relatedness – they change how related interactors are [not by changing anyone's genetics but by changing who interacts with whom (Taylor and Nowak, 2007; Jackson and Watson, 2013)]. How does natural selection act on these traits? For example, initial group size is known to be an important factor in modifying the efficacy of (type 1) group selection (Wilson, 1975; Powers et al., 2009, 2011), and individuals may have traits that modify initial group size (e.g., propagule size) (Powers et al., 2011). The term "social niche construction" refers to the evolution of traits that alter interaction structure, i.e., who you interact with and how much (Powers et al., 2011; Ryan et al., 2016). In some circumstances, natural selection will act to modify such traits toward structures that increase cooperation (Santos et al., 2006; Powers et al., 2011; Jackson and Watson, 2013). This social niche construction has potential advantages over ecological scaffolding because it does not presuppose exogeneous reasons for favourable population structure (that is later canalised by endogenous traits), but shows conditions where such population structure can evolve *de novo*.

Multi-Level Selection and Individuation Mechanisms

In contrast to the kin selection approach (i.e., focussing on the lower-level units and whether they interact with other units that are related), the multi-level selection approach conceives higher-level organisations (collectives) as units of a higher-level evolutionary process (Wilson, 1997; Okasha, 2006; O'Gorman et al., 2008). The multi-level Price approach, for example, attempts to divide the covariance of character and fitness into "between collective selection" (acting at the higher level) and "within collective selection" (acting at the particle level) (e.g., Bourrat, 2021b). Clarke (2016) proposes that we might assess the degree of individuality as "the proportion of the total change that is driven by selection at the higher level," and like Okasha (2006), suggests that an ETI involves a decrease in the proportion of selection driven by the lower level and an increase in the proportion driven by selection at the higher level. In the limit of complete Darwinisation of the collective, and complete de-Darwinisiation of the particles, this becomes maximal.

One problem with this analysis is that, as Wimsatt (1980) points out, the presence of heritable variation in reproductive success at the collective level is not in itself "sufficient for the entity to be a unit of selection, however, for they guarantee only that the entity in question is either a unit of selection or is composed of units of selection." Moreover, Bourrat argues that "there is no fact of the matter as to whether natural selection occurs at one level or another" because "when evolution by natural selection occurs at one level, it does so concomitantly at many other levels, even in cases where, intuitively, these levels do not count as genuine levels of selection" (Bourrat, 2021a). Collectives can be defined at any level and with any boundary, and their character-fitness covariance can be measured, and yet we could have equally well drawn boundaries in any other way. We would have got different quantities (if the interactions among particles are non-linear), but nothing about these quantities tells us how to identify which units are playing a factually causal role in the evolutionary process. Thus, even when there are salient functional interactions among the particles within a collective, it can be hard to disentangle what is happening at one level and what is happening at another, or more exactly, what caused things to happen at one level or another (see also *cross-level by-products* (Okasha, 2006).

Bourrat (2021a) goes on to provide an extension to the multi-level Price approach which divides the *response* to selection (the product of selection and heritability) into a component that is functionally additive (*aggregative*) and a non-additive component. The latter non-aggregative component is associated with the collective response to selection that is not explained by the particle response to selection Thies and Watson (2021). This is useful in drawing attention to the nature of the interactions among particles and its significance in identifying the salient level of causal processes. It also emphasises how a change in *heritability* at the collective level could alter the ability to respond to selection at the collective level. We will develop related ideas below (but argue that in order for higher-level selection to alter evolutionary outcomes, the type of non-aggregative interaction needs to be more specific).

Beyond matters of quantifying individuality, we also aim to better understand the mechanisms that cause these changes (e.g., changes in the ability to produce heritable fitness differences at the collective level) and how selection acts on these mechanisms. In other words, in addition to knowing whether the evolutionary change in a character is explained by lower-level or higher-level evolutionary units, and quantifying how this balance might alter in the course of a transition, we also want to explain how and why this balance changes. We want to explain the mechanisms by which natural selection changes the identity of the evolutionary unit. Here theory is less well developed.

Clarke offers the concept of "individuation mechanisms" that influence "the extent to which objects are able to exhibit heritable variance in fitness" (see also Godfrey-Smith, 2009). These might include developmental bottlenecks, sexual reproduction, egg-eating behaviours, germ separation, immune regulation and physical boundaries (Clarke, 2014, 2016). In general such mechanisms may affect genetic variance (by affecting the extent to which genetic variation is heritable at the collective level), the fitness effects of that variation, or other (non-genetic) sources of heritable variance in fitness. But still, we want to know how selection, more specifically, bottom-up selection, acts on such traits. For example, we need to be able to explain why lower-level selection would act on such traits in a manner that increases non-aggregative components of the collective heritability and response to selection, and not in a manner that decreases it. Intuitively, one might imagine that the reason the traits evolve, the source of their selective advantage, derives specifically from the change in the collective-level response to selection – e.g., the non-aggregative component identified by Bourrat. The models of social niche construction demonstrate that this is possible in some circumstances. However, we cannot assume that it is in the interest of particles to reduce their ability to respond to selection independently, and make themselves dependent on the collective to respond to selection. Given that such traits must be evolved through a particle-level response to selection (since a collective-level response to selection does not exist until after the transition), and that a collective-level response to selection may ultimately create a situation that opposes their direct fitness (e.g., that of somatic cells), this direction of travel is not at all for granted. As yet, these approaches do not tie together the effects that such traits have on the level of individuality with the selection that causes such traits to evolve.

Types of Fitness Interactions: Emergence, Non-aggregative Interactions and Collectives That Change Evolutionary Outcomes

In order for a new level of biological organization to have a meaningful causal role as an evolutionary unit, evolutionary outcomes of the collective must not be simply summary statistics over the lower level units they contain (Okasha, 2006; Bourrat, 2021a). Being a bone fide evolutionary unit requires heritable variation in fitness (Lewontin, 1970; Okasha, 2006), and being a new evolutionary unit (that is "more than the sum of the parts") requires heritable fitness differences at the new level that are not just the average of heritable fitness differences at the lower level (Okasha, 2006). Otherwise, how can it be that collective characters, and not particle characters, determine particle fitness? If particle characters determine collective characters, and collective characters determine the fitness of the particles they contain, then particle characters determine particle fitness. We can write this as follows. If the sum (or other aggregative property) of particle characters (Σz) in a collective determines (linearly) the reproductive output of the collective (Ω), and the reproductive output of the collective determines (linearly) the fitness of a particular particle therein (ω_1), then the value of that particle determines its fitness ($z_1 \rightarrow \omega_1$), and hence the collective is explanatorily redundant in describing the selection on particles (Eq. 1).

$$\left[\sum z \rightarrow \boldsymbol{\Omega} \rightarrow \omega_1\right] \Rightarrow [z_1 \rightarrow \omega_1] \tag{1}$$

The point is perhaps better made by focussing on *changes* in characters and fitnesses. Thus if the change in a character (Δz_1) determines a change in collective fitness ($\Delta\boldsymbol{\Omega}$), and a change in collective fitness determines a change in particle fitness, then changes in particle fitness are determined by changes in particle characters ($\Delta z_1 \rightarrow \Delta\omega_1$), and the collective is redundant.

$$[\Delta z_1 \rightarrow \Delta\boldsymbol{\Omega} \rightarrow \Delta\omega_1] \Rightarrow [\Delta z_1 \rightarrow \Delta\omega_1] \tag{2}$$

So, given that collective characters and hence collective fitness are entailed by the characters of the particles they contain, how can collectives *and not particles* be the reason that one particle character was selected and another was not? The means by which collectives can somehow break the association between particle character and particle fitness will be a key focus of what follows.

To create a meaningful causal role for the collective, there is often an appeal to the notion of creating something qualitatively new at a higher level of organisation, a.k.a. *emergence*. This can be difficult to define (Corning and Szathmary, 2015; Bourrat, 2021a), especially since we generally want to retain the assumption that salient differences at the higher level require salient differences at the lower level (*supervenience*). It is agreed, at least, that in order for the collective to be a meaningful evolutionary unit, fitness interactions between components cannot be linearly additive (Corning and Szathmary, 2015; Bourrat, 2021b). If the fitness-affecting character of the collective is simply the sum or average of the particles, or more generally, an *aggregative* property of the parts (Bourrat, 2021b), then the distinction between higher and lower levels of selection is merely conventional, not substantial (Bourrat, 2021a).

Bourrat examines cases where the relationship between z and collective character, Z (and hence $\boldsymbol{\Omega}$), is non-linear (Bourrat, 2021b). For example, suppose a change in the character of a particular particle (Δz_1) *given a particular context* where the sum of other particle characters has a particular value ($\Sigma z_x = p$), results in a change to collective fitness and hence a change to particle fitness ($\Delta\omega_1$). Now consider the same change, Δz_1, in a different context where the sum of other particle characters has a different value ($\Sigma z_x \neq p$), i.e., we are at a different point on the non-linear curve relating the particle characters to collective character. If this has a different effect on collective fitness ($\Delta\boldsymbol{\Omega}' \neq \Delta\boldsymbol{\Omega}$) and hence a different effect on the fitness of this particular particle ($\Delta\omega_1{}' \neq \Delta\omega_1$) then it does not follow that this change to particle character results in a change in its fitness that is independent of context (Eq. 3). In this sense, the collective is not explanatorily redundant.

$$\left[\Delta z_1 : \left(\sum z_x = p\right) \rightarrow \Delta\Omega \rightarrow \Delta\omega_1\right] \text{and} \left[\Delta z_1 : \left(\sum z_x \neq p\right) \rightarrow \Delta\Omega' \rightarrow \Delta\omega_1'\right]$$

$$\nRightarrow [\Delta z_1 \rightarrow \Delta\omega_1] \text{ nor} \left[\Delta z_1 \rightarrow \Delta\omega_1'\right] \tag{3}$$

Corning and Szathmary (2015) and Bourrat (2021b) describe some examples of possible scaling relationships, such as step functions or thresholds, and super linear curves, that effect a non-linear relationship between the characters of parts and the characters of wholes. The salient criterion of such functions is "whether or not there are combined effects that are interdependent and cannot be achieved by the "parts" acting alone." or "produce an interdependent, qualitatively different functional result" (Corning and Szathmary, 2015).

However, although $\Delta\omega_1{}'$ and $\Delta\omega$ may be different in any such non-linear function, they could nonetheless have the same sign. This is the case whenever the function relating Σz to Z, and $\boldsymbol{\Omega}$, is monotonic (such as a diminishing returns or economy of scale relationship). In this case it will nonetheless be the case that an increase (a particular directional change) in particle character ($\uparrow z_1$) will systematically produce an increase in particle fitness ($\uparrow\omega_1$) regardless of context. That is, for monotonic relationships, the collective is explanatorily redundant in determining *the direction of selection* on particle characters (Eq. 4) (even though the collective character may be non-aggregative).

$$[\uparrow z_1 \rightarrow \uparrow \boldsymbol{\Omega} \rightarrow \uparrow \omega_1] \Rightarrow [\uparrow z_1 \rightarrow \uparrow \omega_1] \tag{4}$$

This means that, although the effect of selection at the collective level may be different from selection at the particle level, it is always affected by particle characters in the same direction. This does not describe cases where higher-level selection changes evolutionary outcomes, i.e, changes in which of two variants are favoured, only how quickly the preferred variant will fix. Such monotonic non-linearities alter only the magnitude of selection, and thus might alter how quickly selection modifies the frequency of a type, but not which type is favoured. Heritable variation in the fitness at the collective level thus remains explanatorily redundant in determining which particle character is favoured by selection.

We think this is not a minor point because altering evolutionary outcomes in this sense – where individual and collective levels of selection "want different things" - is central to ETIs. Restricting attention to monotonic relationships excludes scenarios where the creation of a higher level evolutionary unit

BOX 1 | Non-linearly separable functions.

In machine learning, examples of non-linearly separable functions for two binary inputs are logical exclusive-or (XOR) and if-and-only-if (IFF), meaning that the inputs are different or the same, respectively. In such a function, the contribution of each component input to the output value changes sign depending on the value of another input. For example, if A = true then the output [A XOR B] is made true by B = false. But if A = false then the output [A XOR B] is made true by B = true (for example, *if this cell is soma that cell should be germ*, and vice versa) (**Figure B1**). In functions that are linearly separable (i.e., unitary functions IDENTITY, NOT, and other two-argument functions OR, AND, NAND, and NOR) the effect of an input "shows-through" to the output (or cannot be "decoupled" from the output). That is, if there is a context (a set of values for the other inputs) where increasing a given input increases the output, its effect cannot be the reverse in another context. Put simply, in non-linearly separable functions the sign of the effect of an input on the output depends on an interaction with other inputs. This is a simple way of defining what it means for an output to be non-decomposable or "*more than the sum of the parts*" in a formal sense, i.e., not decomposable into a sum of sub-functions over individual inputs. Technically, the term *linearly separable* refers to the idea that dividing the multidimensional input space into points where the output is true and those where the output is false, only requires one straight line (or, for more than two inputs, one hyperplane). In a non-linearly separable function, in contrast, this is not possible (**Figure B1**). A corollary of this is that linear directional movements through input space can traverse through regions where the output is true, then false, then true again. Put differently, getting from one point where the output is true, to another region where the output is true, without going through a region where the output is false, can require either a nonlinear trajectory or a "jump" in input space where several input variables change simultaneously in a specific manner. It is not guaranteed that there is one variable that, on its own, can be changed incrementally to reach the other region (nor any linear combination of input variables) (see also **Figure B3.B**). This is a simple way to formalise what is meant by a scenario that requires "coordinated action," i.e., variability that maintains a particular output requires specific coordinated simultaneous change in multiple variables.

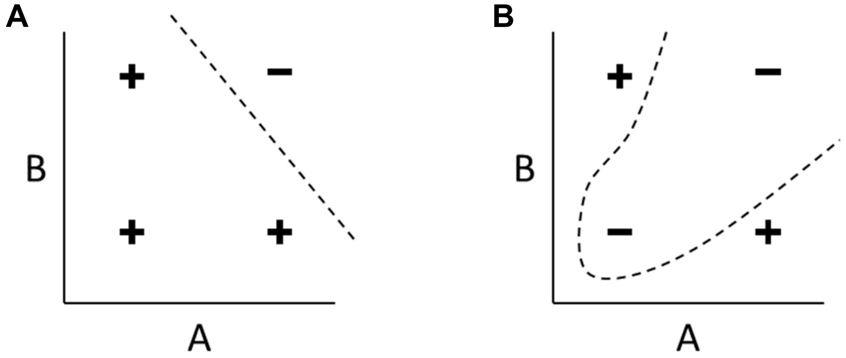

FIGURE B1 | Linearly separable and non-linearly separable functions. **(A)** A linearly separable function of two inputs A and B. The four combinations of high and low values are classified as either positive or negative. In any linearly separable function, like this example representing NAND(A,B), the positive and negative examples can be separated with a linear decision boundary (another example is shown in **Figure B3.B**). **(B)** In any non-linearly separable function, like this example representing XOR(A,B), no such linear decision boundary can be drawn, and separating the two classes requires a non-linear boundary.

causes the lower level units to "do something they didn't want to do" such as evolve characters that decrease their individual fitness (e.g., somatic cells, or other reproductive division of labour), or decrease fitness differences between particles (e.g., fair meiosis, mitochondrial reproductive regulation, or other policing strategies). Although other types of non-linearity where the interaction is not monotonic are sometimes mentioned (in particular a division of labour, as developed below) there is perhaps a reason why the worked examples in previous work have not addressed this. Specifically, if the direction of selection on particle character is different under particle selection and collective selection, such that higher-level selection opposes the phenotypes favoured by lower-level selection, why would bottom-up selection create a new evolutionary unit that opposed its interests in this way?

It is relatively easy to explain why selective conditions can be different (even reversed) after a transition compared to what they were before a transition; as per scenarios of strong altruism, for example. What is not easy to explain is how traits (or the parameters of individuating mechanisms) that change evolutionary outcomes in this way themselves evolve. Before a transition the only entities that can be evolving are particles not collectives, so it must be some character of particles that explains these changes in individuality. How can individual selection favour characters that serve collective interest at the expense of the short-term self-interest of particles?

NEW DATA AND INSIGHTS

A number of current inter-related topics provide new perspectives and new data that contribute to a different way of looking at the evolutionary transitions and individuality.

When the Direction of Selection on Components Is Context Sensitive - Division of Labour Games, Nonlinearly Separable Functions, Non-decomposable Phenotypes, and Comparison With Other Non-aggregative Functions

Intuitively, collectives could alter evolutionary outcomes if the way in which the character of a particle affects the fitness of the particle *depends on the other particles present*. More specifically, the *direction* of selection produced by a change in the character of a particle must depend on the other particles present.

Interactions of this form can be written as follows. Suppose that in one context (say when a neighbouring particle, z_2,

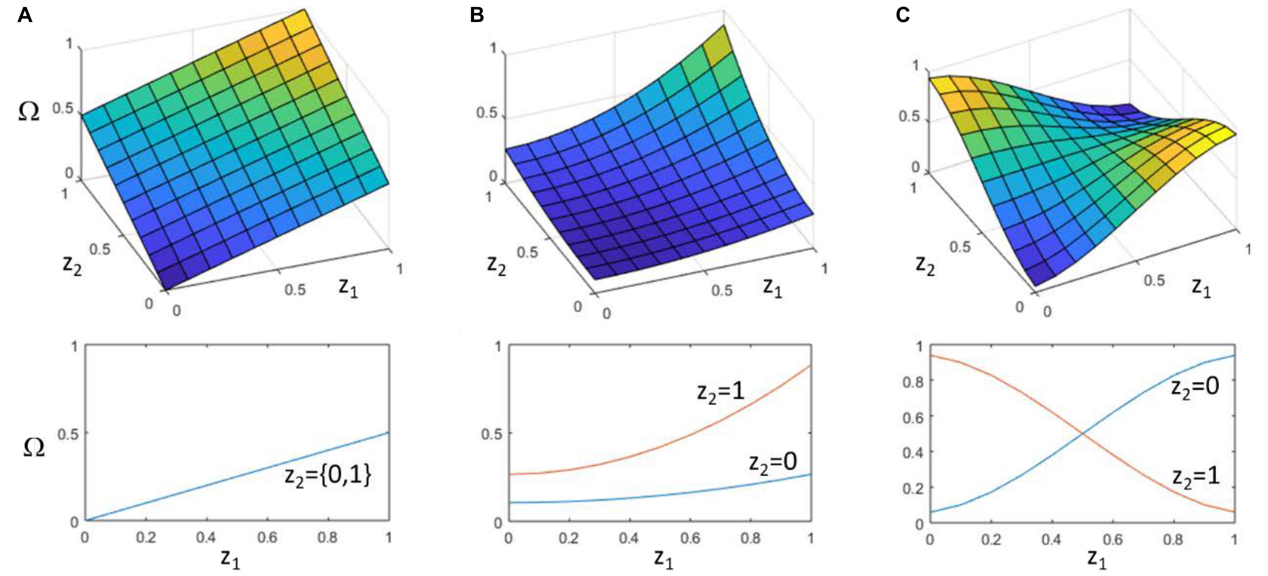

FIGURE 1 | Different possibilities for the relationship of particle characters to collective character. **(A)** *Additive* (no interaction). Two particles, with characters z_1 and z_2, each contribute to collective character, Ω, independently. That is, changes in z_1 always have the same effect on Ω regardless of the value of z_2 and vice versa. **(B)** *Synergistic interaction*. The effect of z_1 on Ω increases with the value of z_2. The two together thus have a synergistic, i.e., super-additive, effect on collective character. **(C)** *Non-decomposable interaction*. The sign of the effect of z_1 on Ω can be reversed by the context of z_2. The class of particle character configurations that confer high values of Ω (yellow) is defined by a non-linearly separable function over z_1 and z_2, such as z_1 XOR z_2 (**Box 1**) (this is not the case in **(B)** which remains linearly separable, i.e., z_1 AND z_2). The lower panels show the value of Ω as a function of z_1. **(A)** insensitive to z_2, **(B)** slope of response depends on value of z_2 but does not change sign, **(C)** sign of response depends on value of z_2. Note that in both **(B,C)**, Ω is a non-aggregative function of the particle characters, but only **(C)** is non-decomposable in the sense of being non-linearly separable.

has a positive character value or a value above a given threshold, θ) increasing particle fitness requires an increase in a particular particle character, and yet in another context (e.g., $z_2 < \theta$) increasing particle fitness requires a *decrease* in the same particle character. In this case, neither an increase nor a decrease in particle character reliably determines an increase in particle fitness (Eq. 5). Accordingly, although collective character determines the direction of selection on particles, particle character does not (Watson and Thies, 2019).

$$[\uparrow z_1 : z_2 > \theta \rightarrow \uparrow \Omega \rightarrow \uparrow \omega_1] \text{ and } [\downarrow z_1 : z_2 < \theta \rightarrow \uparrow \Omega \rightarrow \uparrow \omega_1]$$

$$\nRightarrow [\uparrow z_1 \rightarrow \uparrow \omega_1] \text{ nor } [\downarrow z_1 \rightarrow \uparrow \omega_1] \quad (5)$$

In such cases, the sign of the relationship between particle character and particle fitness depends on what other particles are present. When interacting components are within one evolutionary unit (e.g., genes), this kind of sign change in fitness effects is known as *reciprocal sign epistasis* (Weinreich et al., 2005). But before a transition, the components are different evolutionary units and can instead be construed as players interacting in a game (Hofbauer and Sigmund, 1988). In this case, this kind of sign change in fitness effects is described by a *division of labour* game (Ispolatov et al., 2012; Tudge et al., 2013, 2016), requiring individuals to adopt complimentary heterogeneous roles (Hayek, 1980; Tudge et al., 2013; Watson and Thies, 2019) [e.g., reproductive specialisations such as germ/soma (Godfrey-Smith, 2009)]. The significance of role specialisation and division of labour (or *combination* of labour) in ETIs has been noted by many writers (e.g., Bonner, 2003; Kirk, 2005; Ratcliff et al., 2012; Simpson, 2012; Wilson, 2013; Corning and Szathmary, 2015), but not formally developed in the manner that follows.

In this case, and only in this case, there is no particle character that maximises particle fitness but there is nonetheless a *collective* character (e.g., *complementarity* or *coordination* of particles) that cannot be reduced to the character of individual particles, and this collective character confers (collective fitness and hence) particle fitness. This is a basic but fundamental way of describing a non-decomposable collective character; i.e., a collective character, entailed by particle characters, that confers particle fitness, and yet there is no particle character that systematically confers increases in particle fitness over all contexts.

For what comes later, it will be useful to note that a division of labour scenario is the game theory equivalent of a non-linearly separable function in learning theory (**Box 1**). This provides a formal way to characterise what is important about these functions in evolutionary terms because the distinction between linearly separable and non-linearly separable functions is fundamental in machine learning for the same reasons. That is, the effect of one input changes sign depending on the other input (**Box 1**). We refer to collective characters underpinned by such a function as a non-decomposable collective character (**Figure 1**). That is, the collective character cannot be decomposed into a sum of contributions from individual characters (non-linearity) –

and more specifically, the sign of the effect of changing one particle character is not independent of its context (non-decomposable).

Note that the statistical average of curves in **Figure 1C** can be flat (i.e., the *context-free* contribution of a single particle character to collective character is zero, averaged over all contexts). This does not mean that Ω is insensitive to particle characters; the functional interactions of particles matter significantly in determining the collective character. In this sense, non-decomposability is intimately related to the issue of separating particle character from particle fitness, and the possibility that collective character (and not particle character) determines the fitness of the particles it contains (even though collective character supervenes on particle characters). Note that, confirming the intuition of Okasha (2006), non-decomposability must be defined in terms of traits or characters not particle fitnesses. It is not logically possible for particle *fitness* to control collective fitness and not control its own fitness. But it is possible for particle *traits* to determine collective fitness and not control its own fitness. The particle character matters to its fitness, but the way it matters (the direction of selection conferred by a change in particle character) is not determined by itself independently, i.e., *free from context*.

Note that non-decomposability is a stronger condition than (a refinement of) non-aggregative interactions (Bourrat, 2021b). Non-aggregative interactions include both monotonic non-linear interactions and these non-linearly separable scenarios, but previous examples of non-aggregative interactions have largely been monotonic and thus linearly separable. It is easy to see why: Looked at from the particle level, if a particular change in particle character can increase its fitness in one context and the same change can decrease it in another, how can particles evolve to control and take advantage of collective benefits? Looked at from the collective level, collectives containing an appropriate complement of particle types can solve a division of labour game, and will thus be fitter than a collective that does not. But this creates a problem for the heritability of the collective – *the heterogeneous functions with homogeneous fitness* (HFHF) problem (**Box 2**). To solve this problem, and understand how selection at the lower level can find solutions to non-decomposable problems we need to look at higher-level individuals not as containers of heterogeneous but inert particles, but as dynamical systems that "calculate" collective phenotypes through the interactive behaviours of particles. The domain of such dynamics are the processes of development. How can development perform such computations?

New Perspectives on Organismic Individuality – Development and Basal Cognition

Organismic concepts of individuality, like evolutionary concepts of individuality, can also be hard to pin down (Clarke, 2010; Levin, 2019). Properties such as functional integration, spatial continuity or physical cohesion, coordinated action and developmental dependency, for example, may or may not be aligned with notions of evolutionary or Darwinian individuality (Godfrey-Smith, 2009). Tying individuality to an evolutionary unit identified by its genetics quickly unravels (Godfrey-Smith, 2009; Clarke, 2010). Clonal growth of a bacterial colony may be genetically homogeneous, for example, but does not constitute an organismic individual by most accounts. And even normal looking natural multicellular organisms can be profoundly genetically heterogeneous. For example, planaria are multicellular organisms that can reproduce by fissioning (without a cellular population bottleneck) and thus can accumulate somatic diversity over many generations (Lobo et al., 2012). Nonetheless, planaria exhibit development, morphology and behaviour just like genetically homogeneous multicellular organisms. At a smaller scale, the mechanisms of chromosomal reproduction and (fair) meiosis are tightly coordinated within cells but the chromosomes are genetically heterogeneous. And the behaviour of individual unicellular organisms is, of course, far from a linear combination of gene-products. At a higher level of organisation, holobionts, for example, are sometimes offered as a candidate for a higher-level individual – not because of shared genetics but because of coordinated functional integration and dependencies. Some argue for a view of the biosphere as a whole that is organismic in kind, despite the lack of conditions necessary to be an evolutionary unit. How do we distinguish a collection of multiple organisms that is merely complicated from a new level of individuality?

In multicellular organisms, morphogenesis and its disorder, the breakdown of individuality known as cancer, is intrinsic to individuality (Deisboeck and Couzin, 2009; Doursat et al., 2013; Rubenstein et al., 2014; Friston et al., 2015; Pezzulo and Levin, 2015, 2016; Slavkov et al., 2018; Pezzulo et al., 2021). In most organisms cancerous growths originate from genetically homogeneous tissue and, conversely, in planaria, despite their heterogeneity, cancers are rare. New work shows that cancerous growth can be induced by a disruption of electrical coordination signals between cells and in some cases can be reversed by re-establishing them, without genetic changes (Levin, 2021a). Meanwhile, new experiments demonstrate that artificial multicellular genetic chimera can also exhibit holistic behaviours and functions (Blackiston et al., 2021). These recent experiments and considerations add to the growing evidence that genetic homogeneity is neither necessary nor sufficient for organismic individuality. Is functional integration more important? And what kind of functional integration is necessary and sufficient?

Recent work has begun to apply the tools of collective intelligence and cognitive neuroscience to describe "the signals that turn societies into individuals?" (Lyon et al., 2021a,b). In particular, this includes consideration of behaviours and their reward structures or incentives. Like the considerations of evolutionary individuality above, if the incentives of the whole (its macro-scale reward structures and sensory-action feedbacks) are just summary statistics over the incentives of the parts (micro-scale reward structures and sensory-action feedbacks), then the individuality of the whole is conceptually degenerate. Levin recently makes the case that organismic

BOX 2 | The "heterogeneous functions with homogeneous fitness" (HFHF) problem.

For particle fitness to be determined by collective character and not particle character, a division of labour game is required ("When the Direction of Selection on Components Is Context Sensitive - Division of Labour Games, Nonlinearly Separable Functions, Non-decomposable Phenotypes, and Comparison With Other Non-aggregative Functions"). Solving a division of labour game requires individuals to be different to each other. But if a collective contains multiple types of individuals, how does it reproduce? If reproduction occurs through a single-celled bottleneck or unitary propagule this creates homogeneous descendant groups (and homogeneous groups cannot be solutions to a division of labour game). If reproduction occurs through fissioning the group, or any propagule greater than size one, and individuals are intrinsically different, then selection at the individual level will act on these differences, driving changes in the composition of the group. The latter appears as transmission bias opposing the ability to respond to selection at the higher level (Okasha, 2006). To remove this problem and stop selection at the lower level from interfering with selection at the collective level, the fitnesses of the components must be equalised (de-Darwinised). Individuality thus requires collectives to solve the "heterogeneous functions with homogeneous fitness" (HFHF) problem (Watson and Thies, 2019). Heterogeneous functions are necessary to create fitness differences at the collective level (a.k.a. Darwinisation of the whole); and homogeneous fitness is required to remove fitness differences at the individual level (a.k.a. de-Darwinisation of the parts) (Godfrey-Smith, 2009). But how can particles be functionally different and have the same fitness? Solving the *heterogeneous functions with homogeneous fitness* problem requires individuals to be plastic (Watson and Thies, 2019). This is logical; untying function from fitness requires either plasticity of function or plasticity of fitness. Functional (or phenotypic) plasticity allows individuals to be intrinsically the same (e.g., same genotype and hence same fitness) but act differently (e.g., different phenotype and function). Alternatively, reproductive plasticity (e.g., where reproduction is cued by or enacted by the context of the collective, rather than by autonomous reproductive mechanisms of the particles) allows individuals to be intrinsically different (providing functional complementarity) but reproduce the same (e.g., synchronised reproduction of chromosomes equalises fitnesses) (Watson and Thies, 2019). In evolutionary transitions, these two different ways of solving the HFHF problem are manifest in two different kinds of transitions (Queller, 1997; Watson and Thies, 2019). Fraternal transitions solve the HFHF problem with phenotypic plasticity (and homogeneous genetics) whereas egalitarian transitions utilise reproductive plasticity (and heterogeneous genetics).

individuality is appropriately ascribed to systems that are capable of information integration and collective action at some spatiotemporal scale (regardless of whether they are genetically related or not) (Levin, 2019, 2021b). This is a cognitive notion of "self" ("cogito, ergo sum" perhaps?). But it does not require neurons or brains; *Basal cognition* refers to processes of information integration and collective action that occur in non-neural substrates – such as in the development of morphological form (Pezzulo and Levin, 2015; Manicka and Levin, 2019a,b; Lyon et al., 2021a,b). It refers to cognition in an algorithmic sense that is substrate independent (Levin and Dennett, 2020). "[F]unctional data on aneural systems show that the cognitive operations we usually ascribe to brains—sensing, information processing, memory, valence, decision making, learning, anticipation, problem solving, generalization and goal directedness—are all observed in living forms that don't have brains or even neurons" (Levin et al., 2021). What is important is the presence of functional and informational interactions (signals and responses of any nature) that facilitate information integration and the ability to orchestrate cued responses that coordinate action. In this manuscript we develop this cognitive notion of self by making explicit equivalences with computational models of individuality based on connectionist notions of cognition and learning. This provides the dynamical substrate in which interacting particles can collectively compute solutions that solve the HFHF problem.

Particle Plasticity and Collective Development

Solving the *heterogeneous functions with homogeneous fitness* problem requires individuals to be plastic (Watson and Thies, 2019; **Box 2**). Plasticity allows function and fitness to be separated such that the phenotype of the particle (e.g., whether it is type A or type B) does not determine its reproductive output (Eq. 5). Nonetheless, when this plasticity is used to coordinate phenotypes with other particles, it can access the non-decomposable component of collective fitness. Thus the ability to adopt a phenotype that is complementary to its neighbour (such as "becoming an A when with a B" or "becoming a B when with an A") confers a consistent selective signal (toward being different for XOR, or toward being the same for IFF, **Box 1**). Plasticity thus pushes a collective trait like "diversity" down to a particle trait like "an ability to plastically differentiate." This introduces the notion of a *second order* particle trait – a trait about relationships between things rather than the things themselves – in contrast to a *first-order* or *context free* trait. That is, a second-order trait, such as a differentiating or coordinating behaviour, controls the combinations of *first-order* characters. It is thus an individual character which increases the heritability of a non-decomposable collective character (e.g., phenotypic diversity necessary to solve a division of labour game).

Note that although the direction of selection on a first-order individual character will reverse depending on context in a division of labour game, the direction of selection on the second-order character (e.g., favouring being different rather than being the same) is consistent for a given game. It is then possible to attribute particle fitness to this (second-order) particle character. This appears to put us back at square one with a collective that is explanatorily redundant (Eq. 4). But note that second-order characters such as plasticity really are different from first order characters because they are about relational attributes. For example, a particle cannot be "the same" or "different" on its own, and a phenotype that is sensitive to the context of others cannot be assigned a fitness until the others are present and the plasticity is enacted (i.e., development happens). Intuitively, although the property of being able to plastically differentiate from your partner is a property that a single particle can have, the ability to solve a division of labour game is not a property that a single particle can have. This collective property is the result of a basal "calculation" performed by multiple particles within the collective in interaction with each other. When this functional outcome (a solution to the division of labour game) is a non-linearly separable function of the individual particle characters, the fitness of the particles (and more specifically, the direction of selection on particle characters) that results cannot be attributed to those individual particle characters, and

BOX 3 | Depth is required to represent non-linearly separable functions.

In simple artificial neural networks (e.g., the Perceptron), the output of each neuron is a function of the sum of its weighted inputs (Minsky and Papert, 1988). The shape of this function is non-linear but monotonic (e.g., sigmoidal or threshold). For a single neuron, a particular input might influence the output more or less strongly than other inputs (depending on the magnitude of the weight), it might have a positive or negative influence (depending on the sign of its weight), and because of the non-linearity of the output function, the slope of this influence can be affected by its magnitude and the magnitude of other inputs. But the influence of a particular input on the output cannot change sign. Whether it increases or decreases the output is not sensitive to other inputs [by analogy, see the difference between magnitude epistasis and sign epistasis (Weinreich et al., 2005)]. This means that when directional changes in the input "show-through" to directional changes on the output they do so in a consistent manner, i.e., there cannot be two contexts where a given change on the input has the opposite effect on the output. This property makes it easy to incrementally adjust the weights toward a desired output function because the correct direction to change a weight does not depend on the state of other inputs. However, this means that a single neuron of this type, or a network with a single layer of such neurons, cannot compute non-linearly separable functions of the inputs, where the influence of one of the inputs must be reversed depending on the value of the other input (**Box 1**). To represent a non-linearly separable function an intermediate level of representation (or "hidden layer") between inputs and outputs can be employed. A multi-layer Perceptron can compute A XOR B, for example, by computing OR(AND(A, NOT(B)), AND(NOT(A),B)), i.e., (A XOR B) = "A without B or B without A." The sub-functions used in this construction (AND, OR, and NOT) are all linearly separable functions (computable with a single Perceptron). One node in the hidden layer, let's call it ***h1***, can thus compute ***h1*** = AND(A,NOT(B)) and another node can compute ***h2*** = AND(NOT(A),B), and then an output node can be stacked on top to compute OR(***h1***,***h2***). More generally, to represent a non-linearly separable function, a network must be able to compute higher-order or multiplicative terms – not just a weighted sum of inputs.[1]

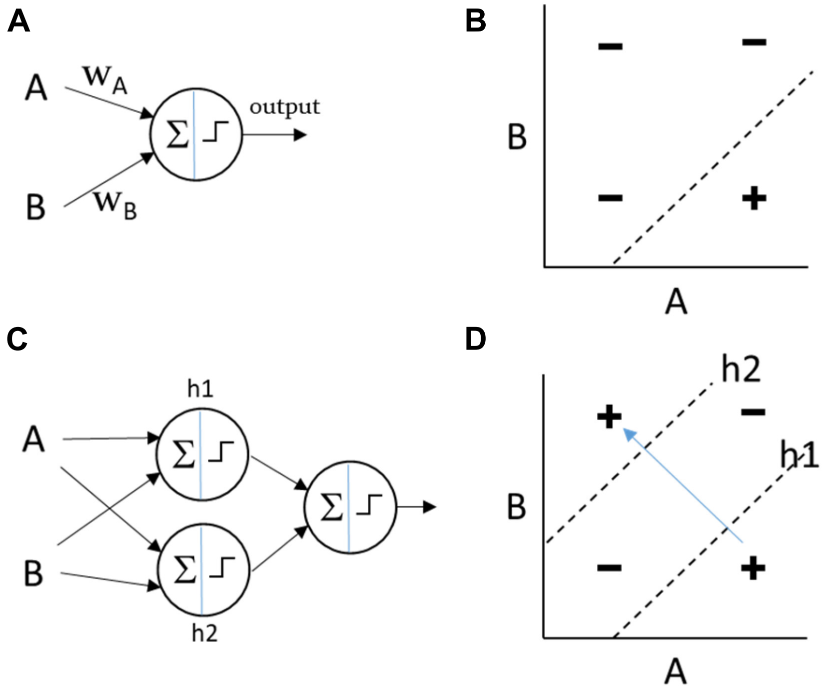

FIGURE B3 | Shallow and deep computations. **(A)** A Perceptron of two inputs calculates an output that is a non-linear weighted sum of its inputs. **(B)** The Perceptron can represent any linearly separable function, such as this example, AND(A,NOT(B)). **(C)** The multi-layer Perceptron utilises "hidden" nodes to calculate intermediate functions which are fed forward to the output node. This can calculate any linearly or non-linearly separable function of its inputs. **(D)** In this example, h1 calculates AND(A,NOT(B)) and h2 calculates AND(NOT(A),B). The output node can calculate OR(h1,h2) such that the network as a whole represents the non-linearly separable function XOR(A,B). In a non-linearly separable function, moving between different positive regions (variation within the class without visiting regions that are not in the class) cannot be achieved by linear movements in the input space and instead requires "jumps" or coordinated "collective action" (simultaneous discontinuous changes in multiple variables).

accordingly, the collective is not explanatorily redundant. This view thus resolves the tension between the two desiderata of (i) collectives that are not explanatorily redundant and (ii) collective properties that are nonetheless determined by particle properties.

We thus identify particle plasticity (enabling coordinated phenotypes or coordinated reproductive behaviour between particles) as a concrete type of individuation mechanism. This is a particularly significant type because it enables access to components of selection that cannot be otherwise be accessed precisely when functional interactions between particles have a non-decomposable relationship. Because the ability to coordinate with others is a characteristic that can be heritable at the particle level, and the result of this ability is a coordinated collective phenotype that would not otherwise be heritable, this facilitates a response to selection at the collective level that was not previously present. This particular kind of particle-level trait therefore

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[1] This could be provided by a non-monotonic output function (where, for example, over-saturation of inputs depresses outputs) – but this would make it impossible to represent ordinary linearly separable relationships with the same network. Alternatively, multiplicative interactions could be implemented by synaptic connections that mediate the sign of other synaptic connections directly, e.g., via axoaxonic synapses that join directly with another incoming connection rather than the dendrites of the downstream neuron.

connects directly with the particular kind of non-aggregative component of selection, and the collective level heritability, required to facilitate a response to selection at the collective level (Bourrat, 2021b).

How does the necessary plasticity evolve? Given the consistent direction of selection on plastic traits, Tudge et al. (2016) showed that natural selection can evolve phenotypic plasticity that solves division of labour games in two-player collectives with homogeneous genotypes, by evolving phenotypic sensitivity to one-another to facilitate complementary differentiation (Brun-Usan et al., 2020). Plasticity of any kind requires a timescale on which it can take effect – time to go from undifferentiated types (genotypes) to differentiated types (phenotypes), with communication between one particle and another to determine the coordinated outcome. In a fraternal transition, this temporally extended process effects a minimal separation between an "embryonic group" (undifferentiated components with the same genotype) and the "group phenotype" (differentiated components with coordinated complementary functions) – and the process that separates them is a minimal model for *development*.[2] To Darwinise the collective at the same time as de-Darwinising the components thus requires the components to be plastic and a developmental process that coordinates their behaviour. The Tudge model, involves just two particles and the one connection between them. It also assumes genetic relatedness which presupposes the higher-level unit of selection and its heritability. The evolution of relationships that solve the HFHF problem in more general networks of interactions (more than two players, thus more general games), and under bottom-up selection, has not yet been shown.

Note that development is not merely a process that modifies particle phenotypes and particle fitnesses, but more specifically, to produce fitness differences that properly belong to the collective level, it must solve a division of labour game. These considerations argue that developmental interactions required for evolutionary individuality must be able to coordinate solutions to non-decomposable functions of this type. This complexity exists in the substrate of basal cognition (implicated in organismic individuality) and at the timescale of organismic development. It suggests that organismic individuality (i.e., the plasticity of particles, and the developmental interactions that coordinate their differentiation) is intrinsic to Darwinian individuality (i.e., creating non-decomposable fitness differences that properly belong to the collective level). Recent expansions on the equivalence between evolution and learning provide a new theoretical framework to make sense of and unify these observations. In particular, these develop connectionist models of cognition and learning that focus on interactions (or second-order characters) in systems of many components and many interactions.

Connectionist Models of Cognition and Learning

Connectionism explores the idea that the intelligence of a system lies not in the intelligence of its parts but in the organisation of the connections between them. Each neuron might be computationally trivial (e.g., a unit that produces an output if the sum of its inputs is strong enough), but connected together in the right way, networks of such units have computational capabilities at the system level that are qualitatively different. For example, the output of a network can be a non-linearly separable function of its inputs (**Box 3**), and built-up in multiple layers (the outputs of one layer being the input to the next), such networks can represent any arbitrary function of its inputs. In networks with recurrent connections (creating activation loops), the system as a whole can have multiple dynamical attractors that produce particular activation patterns. The information that produces these patterns is not held in any one neuron (or any one connection) but in the organisation of the connections between them. Patterns stored in this way can be recalled through presentation of a partial or corrupted stimulus pattern, known as an "associative memory" (Watson et al., 2014; Power et al., 2015).

System-Level Organisation Without System-Level Reinforcement

The organisation necessary for such distributed intelligence can arise through simple learning mechanisms – without design or selection. In most learning systems, the learning mechanism (used to adjust connections) is simply incremental adjustment that follows local improvements in an objective function. The objective function can be based on the accuracy of the output (supervised learning), the fit of the model to data (unsupervised learning), or the reward from behaviours that are generated from the model (reinforcement learning).[3] Supervised learning requires an "external teacher" to define a desired output or target but reinforcement learning only requires a "warmer/colder" feedback signal and nothing more specific (reinforcement learning is commonly identified as the analogue of evolution by natural selection, but for bottom-up evolutionary processes we are particularly interested in unsupervised learning (Watson and Szathmary, 2016). Unsupervised learning does not depend on a reinforcement signal at all. It demonstrates conditions where the organisations necessary to produce system-level cognitive capabilities can arise through very simple distributed mechanisms operating without system-level feedback. A simple example is the application of Hebbian learning often paraphrased as "neurons that fire together wire together" (Watson and Szathmary, 2016). This mechanism changes relationships (under local information, i.e., using only the state of the two nodes involved in that connection) in a manner that makes the connection more compatible with the current state of the nodes it connects. Despite this simplicity, this type of learning is sufficient to produce an associative memory capable of

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[2] In an individual resulting from an egalitarian transition, the language would be different. For example, this temporally extended process of interaction might be called collective or group reproduction, rather than development. That is, an embryonic group containing intrinsically different components (with different functions and different genotypes) is coordinated by these processes to produce undifferentiated component-reproduction (coordinated and identical reproductive opportunity).

[3] And may include regularisation terms that apply or modify an inductive bias, as discussed in section "System-Level Optimisation Without System-Level Reinforcement."

storing and recalling multiple patterns, generalisation, data-compression and clustering, and optimisation abilities ("System-Level Optimisation Without System-Level Reinforcement").

Learning is not the same as simply remembering something. Learning (apart from rote learning) requires *generalisation* – the ability to use past experience to respond appropriately to novel situations. That is, the ability to model (recognise, generate or respond to) not just the situations encountered in past experience but also novel situations that have not been encountered before. Connectionist models of cognition and learning exhibit generalisation naturally. When representing the pattern "11," for example, the network could represent that *the first neuron value is "1,"* and independently, *the second neuron value is "1."* But because networks can represent patterns with connections, it can also represent an association between the value of neuron 1 and the value of neuron 2 – in this case, that *the values are the same*. This "associative model" represents not just this particular pattern but the class of patterns where the values have the same relationship. In this example, it will also include "00." In some situations, this might be a mistake – after all, "00" has no individual values in common with "11." But the relationships between values (such as "sameness" or "differentness") in a pattern are higher-order features that might represent useful underlying structures within a broader set, or "class," of patterns. If consistent with past experience, learning such relationships enables generalisation that cannot be provided by treating individual components of the pattern as though they were unrelated. This enables neural networks, over an extraordinarily broad range of domains, to learn generalised models that capture deep underlying structural regularities from past experience and exploit this in novel situations.

System-Level Optimisation Without System-Level Reinforcement

Because of their ability to generalise, neural networks can also discover novel solutions to optimisation problems. Specifically, simple fully-distributed mechanisms of unsupervised learning, using only local information, can produce system-level optimisation abilities (Watson et al., 2011a,c). The initial weights of the network define the constraints of a problem and running the network from random initial states finds state patterns that correspond to locally optimal solutions to these constraints (Hopfield and Tank, 1986; Tank and Hopfield, 1987a,b). If the network is repeatedly shocked or perturbed, e.g., by occasionally randomising the states, with repeated relaxations in between, this causes it to visit a distribution of locally optimal solutions over time. Without learning, however, it cannot learn from past experience and may never find really good solutions. In contrast, if Hebbian learning slowly adjusts the weights of the network whilst it visits this distribution of locally optimal solutions, the dynamics of the system slowly changes. Specifically, these systems learn to solve complex combinatorial problems better with experience (Watson et al., 2009, 2011a,c). This is because the network learns an associative model of its own behaviour [known as a *self-modelling dynamical system* (Watson et al., 2011c)]. That is, it forms memories of the locally optimal solutions it visits, causing it to visit these patterns more often in future. This is because Hebbian changes to connections have the effect of creating a memory of the current state, making it more likely that the system dynamics visits this state in future by increasing its basin of attraction (i.e., the region of configuration space that is attracted to that state configuration by the state dynamics). Moreover, because it is an associative model, it is not simply memorising these past solutions but learning regularities that generalise. That is, any state configuration that shares that combination of states (consistent with that connection) is more likely to be visited. This means it also enlarges the dynamical attractors for other states it has not visited in the past but have similarly coordinated states. Over time, as relationships change slowly, the attractor that is enlarged the most tends to be a higher quality solution, sometimes even better than all of the locally optimal attractors visited without such learning (Mills, 2010; Watson et al., 2011a,b,c; Mills et al., 2014). The ability to improve performance at a task with experience is perhaps not unexpected in learning systems. But important for our purposes here, there is no reinforcement learning signal used in these models – system-level optimisation is produced without system-level feedback, using only unsupervised and fully-distributed Hebbian learning acting on local information, and this repeated perturbation and relaxation.

Furthermore, the principle of Hebbian learning is entirely natural; it does not require a mechanism designed or selected for the purpose of performing such learning. Specifically, Hebbian changes to connections result from incremental "relaxation" of connections, i.e., changes that reduce conflicting constraints, reduce the forces that variables exert on one another, or equivalently, decrease system energy (Watson et al., 2011a,c). This means that any network of interactions, where connections differentially deform under the stress they experience, can exhibit this type of associative learning and optimisation. The action of natural selection provides one such case in point when there is heritable variation in connections – even without system-level selection. This enables the computational framework of cognition and learning familiar in connectionist models to be unified with the evolutionary domain – hence *evolutionary connectionism*.

Evolutionary Connectionism

Evolutionary connectionism is a new theoretical framework which formalises the functional equivalence between the evolution of networks and *connectionist* models of cognition and learning (Watson et al., 2016; Watson and Szathmary, 2016). This work shows that the action of random variation and selection, when acting on heritable variation in relationships, is equivalent to simple types of associative learning. Accordingly, these models can be translated into the domain of evolutionary systems to explain the evolution of biological networks with system-level computational abilities (Watson et al., 2010; Kounios et al., 2016; Kouvaris et al., 2017; Brun-Usan et al., 2020). This work demonstrates mechanisms of information integration in biological interaction networks, equivalent to simple (but powerful) types of neural network cognition.

In some cases, these models characterise the evolution of developmental organisation (evo-devo) where the interactions are inside a single evolutionary unit (among the multiple components it contains), such as gene-regulatory interactions (Watson et al., 2010). The kind of information integration that gene-networks can evolve is the same as that which neural networks can learn and, for example, is capable of demonstrating associative memory (one genotype can store and recall multiple phenotypes, recalled from partial or corrupted selective conditions) and generalisation (networks can produce novel adaptive phenotypes that have not been produced or selected in past generations) (Watson et al., 2010; Kouvaris et al., 2017). These models demonstrate that the conditions for effective learning can be transferred into the evolutionary domain and help explain biological phenomena such as the evolution of evolvability (Kounios et al., 2016; Watson, 2021). However, these models assume that selection is applied at the system level (equivalent to reinforcement learning at the system level).

System-Level Organisation Without System-Level Selection: Evolving Organised Relationships Bottom-Up

For the ETIs, driven by bottom-up selection, we cannot assume a reward function that operates over the system as a whole; rather it must be analogous to a reward function for each individual particle (Power et al., 2015). How does reinforcement learning at the level of individual particles, in interaction with each other and acting on their relationships, change system-level behaviours?

Previous work shows that the action of fitness-based incremental change at the individual level (or individual reinforcement learning in a network of pairwise games (Davies et al., 2011; Watson et al., 2011a), when applied to relationships between agents, is equivalent to unsupervised associative learning at the system scale. That is, *individual-level reinforcement learning*, when given control over the strength of connections, is equivalent to *unsupervised* learning at the system level (Davies et al., 2011; Watson et al., 2011a; Power et al., 2015). This means that the same learning principles can be translated into evolutionary scenarios where the system is not a single evolutionary unit but a network of relationships among many evolutionary units – such as an ecological community with a network of fitness dependencies between species. These models characterise the evolution of ecological organisation (evo-eco) under individual-level natural selection (Power et al., 2015; Watson and Szathmary, 2016). Even though, in this case, selection acts at the level of the components not at the system level, the kind of information integration that community networks can evolve is also the same as that which neural networks can learn (with unsupervised learning) (Power et al., 2015). These learning principles do not depend on any centralised mechanisms, or an external teacher/system-level feedback (Watson and Szathmary, 2016). This can be used to demonstrate the evolution of ecological assembly rules that implement an associative memory that can store and recall multiple ecological attractors that have been visited in the past and recall them from partial or corrupted ecological conditions (Power et al., 2015). This is crucial in demonstrating how natural selection organises interaction networks bottom-up - *before* a transition.

System-Level Adaptation Without System-Level Selection: Bottom-Up Adaptation

Under suitable conditions, these models also demonstrate non-trivial problem-solving optimisation at the system level without system-level selection. As in the analogous neural systems ("System-Level Optimisation Without System-Level Reinforcement"), when the initial connections between individuals constitute a system of random constraints (or pairwise games), running the network to an attractor (i.e., repeatedly allowing all individuals to make their own decisions about the state that maximises their individual utility) increases total utility. Intuitively, each unit is incentivised to maximise their individual utility (by definition) and if each of them acts to increase their individual utility then the total utility tends to increase as well (Davies et al., 2011; Watson et al., 2011a,c) (this is guaranteed if the interactions are symmetric). However, because of the conflicts and constraints between individual incentives, the short-sightedness of their actions and the fact that individual behaviours have no system-level incentive to maximise the utility of others, the attractors found are only *locally* optimal (again, as analogous to the neural systems). Other attractors may exist with higher total utility but these are only found if the system happens to start from very specific initial conditions (Watson et al., 2011a).

When individual reinforcement or selection is allowed to modify the strength of the relationships between units, the system becomes a self-modelling dynamical system and its dynamics change in predictable ways, as per the distributed optimisation shown in neural models. Specifically, if the state of the system (species densities) is repeatedly shocked or perturbed, causing it to reset to different random initial conditions and repeatedly allowed to relax into different ecological attractor states, the relationships that evolve enlarge the dynamical attractors for the distribution of locally-optimal states visited. Because selection is changing the relationships between species, and associative learning can generalise, it also enlarges the dynamical attractors for other states with even higher total utility. The evolution of interactions in an ecological community can thus produce adaptive organisation at the network level without presupposing that the network is an evolutionary unit.

To provide a compelling example of what this can do, Power sets up the initial competitive ecological interactions between species to represent the constraints of a resource allocation problem equivalent to a Sudoku puzzle (Power, 2019). The profile of species densities represents assignments of numbers in a Sudoku solution, and the community matrix of fitness dependencies between them represents the rules of the puzzle (e.g., two "6"s in the same row, column or box have a strong competitive interaction). Running the initial Lotka-Volterra ecological dynamics from random initial species densities finds one of very many ecological attractors corresponding to, generally poor, locally optimal solutions (i.e., with many constraints violated). Power then showed that individual-level natural selection, acting on traits that affect inter-specific interactions, caused the attractors of the ecological dynamics to change, and showed conditions where this causes the community to form attractors that correspond to better quality solutions over evolutionary time (Power, 2019). Under these conditions, the resultant ecosystems, evolving without any system-level selection, can in many cases learn to solve Sudoku puzzles that humans find very difficult to solve.

Some comparisons with ecological scaffolding are notable. Both this effect and ecological scaffolding utilise the observation that, when a system is held in a particular state, the action of natural selection on the components therein is likely to reinforce that state – making a "memory" of that configuration. In ecological scaffolding this means that the scaffolding conditions can be removed and the organisation persists, in ecological memory (Power, 2019), the system state can be perturbed and it will, with greater probability, return to this state. In both cases this results in a system that adopts configurations of higher-total utility or higher cooperation than it would otherwise. Some important differences are that in scaffolding the one new state is (initially) created by exogenous conditions that oppose the natural attractors of the system, causing it to adopt states that are conducive to more cooperation, whereas in Power's model of ecological adaptation, the system visits many states that are each natural local attractors, and no exogenous conditions need be changed. In scaffolding the canalisation of the new state can be any evolutionary change that maintains that state (e.g., changes to population structure that restrain the interactions in the same way), here we are interested more specifically in associative relationships that have the capability to represent underlying structural regularities that generalise over the set of states visited. This is important because (a) in scaffolding, the state that is initially imposed by exogenous factors is the state that is ultimately canalised. Insomuch as the exogenous imposition of ecological factors is not in itself an adaptive process, whatever outcome it produces is fortuitous happenstance. Though its results might have adaptive consequences, it does not require an adaptive explanation. (b) In contrast, in the effect described by Power, the ultimate outcome is a novel state that the system finds by an adaptive process of generalisation. This is a true optimising effect, explained by selection from below, not fortuitous happenstance. Nonetheless, in general cases, there is plenty of scope for exogenous ecological scaffolding, this effect, and others to interact with one another in complex ways.

Evolutionary connectionism thus translates distributed learning principles into the domain of natural selection, and demonstrates how relationships among evolutionary units can become adaptively organised by selection on the existing, lower-level units – or more exactly, on the characters of lower-level units that affect the relationships between them. This thereby demonstrates conditions where multiple short-sighted, self-interested entities organise their relationships with one another causing them to act in a manner that is consistent with long-term collective interest – increasing total welfare (the sum of individual fitnesses). Individuals do not do this because they are intrinsically motivated by long-term or pro-group interests (they are short-sighted and self-interested), but under these conditions, short-term self-interest acting on slow-changing relationships between individuals (second-order traits) produces this systematic outcome. The organisation of the whole becomes conditioned by its past experience, with distributed incremental changes to its organisation motivated to reduce individual-level conflict, and because this occurs over a distribution of many ecological equilibria, each resolving some subset from the same set of conflicting constraints, it generalises from this past experience to influence future behaviour in a manner that resolves more of these conflicts (Watson et al., 2011a,b; Power, 2019).

Thus far, however, these models have not demonstrated transitions in individuality. In the models of gene regulation networks, the evolutionary unit was already at the network level (evo-devo). In the models of ecological dynamics, the evolutionary unit was at the lower (individual) level (evo-eco) and although there are observable fitness consequences at the network level, the ecological community does not become a new reproductive unit with heritable fitness differences, nor is there a de-Darwinisation of particles. Neither model demonstrates a change in the level of individuality, or "evo-ego" (Watson and Thies, 2019). What is missing?

HYPOTHESIS AND THEORY: TOWARD A CONNECTIONIST FRAMEWORK OF INDIVIDUALITY

The framework of *evolutionary connectionism* provides a basis on which to develop a different kind of theory for ETIs. Conventional evolutionary thinking suffers the chicken-and-egg problem of transitions because it attempts to explain adaptations through changes to the frequency of units, which presupposes an evolutionary unit (at the relevant level) is already defined. In contrast, a connectionist approach explains adaptations through the changing organisation of the relationships between existing lower-level units. This provides a way for the whole to become more than the sum of the parts, in a formal sense, without presupposing that the whole is already an evolutionary unit. It is a theory that focusses not on the things (and their frequencies) but on the relationships between things (and the transformation of their organisation). Connectionism provided a way for cognitive science to escape the infinite regress of homoncular thinking (i.e., the whole is intelligent only because it is composed of intelligent components), and showed that the whole can have cognitive abilities of its own (more than the sum of the parts), even though the individual components are cognitively trivial, *if* the relationships between them are organised appropriately. Here we aim to translate this into the evolutionary domain to provide a way for evolutionary theory to resolve the chicken-and-egg problem of individuality, and show that the whole can have individuality of its own (more than the sum of the parts), even though the components are self-interested (have no foresight or pro-social assumption), *if* the relationships between them are organised appropriately.

> *But How Exactly Do Relationships Need to Be Organised to Produce a New Level of Individuality?*

What kind of interaction structures turn a society into an individual? (Lyon et al., 2021a,b). Here we develop the hypothesis that translating further principles of connectionist cognition and learning into the domain of evolutionary systems describes both the specific kind of relationships that are needed for an ETI and the conditions under which they can evolve through bottom-up selection.

Design for an Individual

Summarising the conditions discussed above, in order for an evolutionary unit to be meaningful it must explain evolutionary outcomes that cannot be explained by the summative effects of the components it contains. Collective characters must therefore be non-linearly separable functions of (embryonic) particle characters ("When the Direction of Selection on Components Is Context Sensitive - Division of Labour Games, Nonlinearly Separable Functions, Non-decomposable Phenotypes, and Comparison With Other Non-aggregative Functions"). In order for particles to effect such collective characters, particles must be plastic (either phenotypically or reproductively) ("Particle Plasticity and Collective Development"). Plasticity allows for the phenotype or the reproduction of a particle to *not* be determined by the intrinsic independent properties of the particle but rather by its interactions with other particles (e.g., their coordination or complementarity). Before a transition these interactions are ecological (i.e., between multiple evolutionary units) and after the transition these same interactions are developmental (i.e., among the components of a single evolutionary unit) (Watson and Thies, 2019; Fields and Levin, 2020). The dynamical process controlled by these interactions is what we recognise as the basal cognition of development - implementing information integration (computing a non-decomposable function of input states) and collective action (producing specific coordinated responses in multiple downstream variables). Ultimately, this collective action must control the reproduction of the particles involved (such that their fitness is determined by collective-level properties – properties that cannot be decomposed into the properties of the individual particles). This might result in synchronised reproduction or reproductive specialisation (the egalitarian or fraternal solutions to the HFHF problem, respectively).

Evolutionary individuality thus requires a developmental process that constitutes the computation of a non-linearly separable function (between "embryonic" collections of particles and "adult" collective phenotypes) ("Particle Plasticity and Collective Development"). When evolved interaction structures between units compute a function that is non-linearly separable, this makes collective fitness, and hence reproduction, non-decomposable in this formal sense. Natural selection, acting bottom-up, can modify relationships between units in a manner that creates adaptive organisation at the system scale. So, perhaps it might create interactions that compute the non-linearly separable functions required for a transition in individuality? However, shallow or single-level interaction structures cannot compute non-linearly separable functions (**Box 3**). Previous models only allowed for the evolution of shallow interaction structures – a single layer of symmetric, all-to-all relationships (e.g., a↔b, b↔c, c↔a). In order for a system to compute non-linearly separable functions, the interaction structure must have some depth[4] (**Box 3** and **Figure 2**).

The significance of deep structure is demonstrated by further work with neural networks. An adaptive process with the ability to learn and exploit deep structure has different adaptive capabilities from an adaptive process that cannot – this is referred to as *deep optimisation* (Caldwell et al., 2018, 2021), or *multi-scale search* (Mills, 2010; Mills et al., 2014). In neural models this affords new levels of variation (coordinated action) that can access new levels of reward structure (higher-order epistatic components of fitness) (Watson et al., 2011b), i.e., coordinate changes to multiple units simultaneously. This progressive hierarchical abstraction, with each higher-level of representation building on the representations of the layer below, is familiar in machine learning and, we argue this is analogous to the way in which ETIs enable *deep biological evolution* (i.e., multi-scale evolutionary processes) to implement deep model induction (Mills, 2010; Mills et al., 2014; Watson and Szathmary, 2016; Czégel et al., 2018, 2019; Vanchurin et al., 2021). In the machine learning context we have shown that this coordinated collective action can find high-quality solutions to combinatorial optimisation problems that cannot be accessed by individual action (Watson et al., 2011b; Caldwell et al., 2018, 2021).

This suggests that (a) the interaction structures necessary for evolutionary individuality, (b) the interactions structures necessary for organismic individuality, and (c) the interaction structures required to compute non-linearly separable functions are intimately related. Specifically, when interactions among evolutionary units form collective phenotypes that are non-linearly separable functions of their embryonic phenotypes, and this integrated information then cues behaviours that coordinate the reproduction of the particles, this constitutes a new level of evolutionary individuality. In order for such interactions to compute non-linearly separable functions, the interaction structure cannot be shallow or reciprocal (as in previous models) but must have some depth. We thus describe a view of evolutionary processes where, given appropriate conditions, interaction structures will evolve (through bottom-up selection) in a way that "mirrors" structure in the selective environment (Wagner and Laubichler, 2004; Kounios et al., 2016; Kuchling et al., 2020) – and that when this structure is deep, this constitutes a transition in individuality (**Figure 2**).

This leads to our main hypothesis about the architecture of individuality:

> *H1. Individuality requires a dynamical process (development), mediating the plastic expression of components in the context of one another, with the specific form of computing a collective character that is a non-linearly separable function of (embryonic) particle characters, with the effect of coordinating reproduction based on this collective character.*

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[4] In modern neural networks, "deep" is often used to mean that there are very many computational layers, sometimes hundreds. Here we only mean that the computation cannot be single layer (simply connecting inputs to outputs directly), but must (minimally) include connections that go from inputs to outputs via hidden state variables.

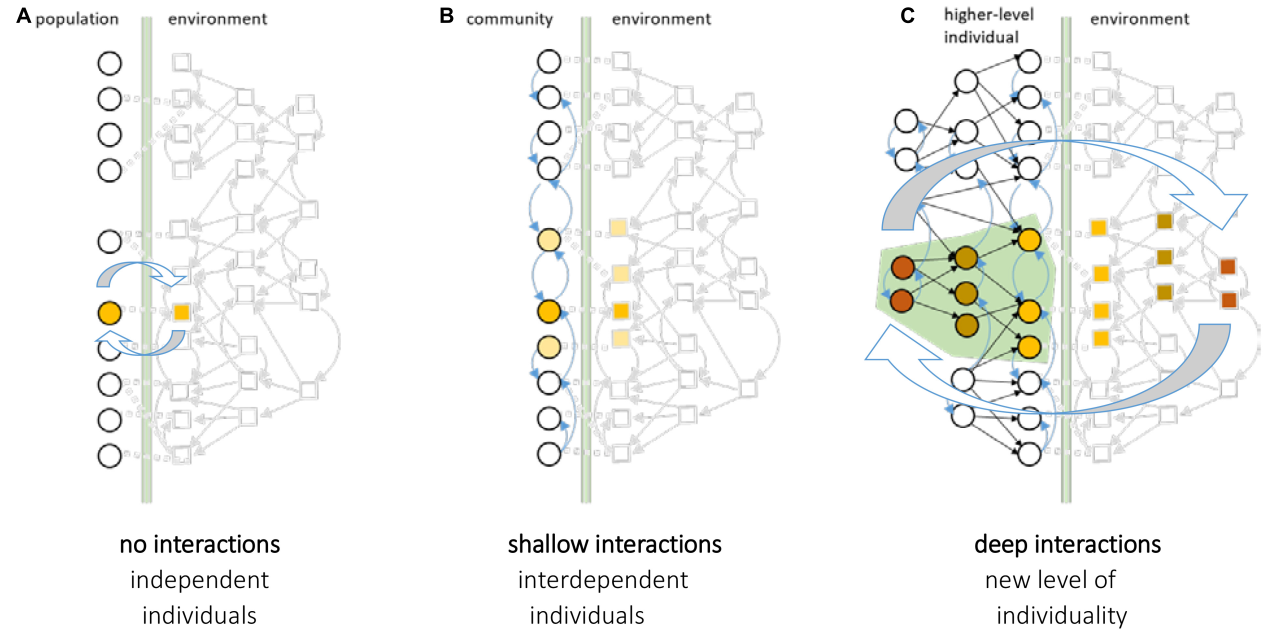

FIGURE 2 | Overview of conventional models of evolution (A), previous work in evolutionary connectionism (B) and Hypothesis 1 (C). **(A)** A conventional view of evolution models individual evolutionary units in a population. The complexity of the environment is not "visible" to evolution – selection on an individual (orange circle) responds only to corresponding additive effects of selection in the environment (orange square) (large arrows). At the population level, this is formally equivalent to simple (model-free) forms of learning and inference (Frank, 2009; Harper, 2009; Shalizi, 2009). **(B)** When individual traits affect behavioural or phenotypic interactions with other individuals, these relationships can evolve to "mirror" dependencies among environmental variables. When interactions are reciprocal (single-level) the evolution of the community is formally equivalent to shallow models of connectionist learning (Watson et al., 2014, 2016; Power et al., 2015; Kouvaris et al., 2017). **(C)** We hypothesise that when functional interactions between individuals in a community are directional, creating deep interaction structures with "hidden" variables, evolution is formally equivalent to deep model induction. When evolved functions are non-linearly separable, and their outputs control the coordination of particle reproduction, the hierarchical structures so created constitute new individuals at a higher level of organisation (e.g., green). The functional interactions between component individuals constitute a developmental process for this higher-level individual, and enable information integration and collective actions at a new level of biological organisation. Adaptations under natural selection (acting on deep variables, red circles) now serve the development, survival and reproduction of the new individual (large arrows), and deep causal variables in development (red circles) mirror deep causal variables in the selective environment (red squares).

Only under these conditions, we hypothesise, can it be true that multiple individuals have relationships that cause them to work together for long-term collective benefit despite causing behaviours that oppose their short-term individual interest. On the fine timescale we call development, we might observe this as delayed or prohibited individual reproduction of some cells within a multicellular organism, for example. Whereas on the longer timescale relevant to the reproduction of the collective, we might observe this as a coordinated or specialised reproductive behaviour that affords access to higher-order fitness differences by allowing information integration and collective action. This can be directed at the control of reproductive plasticity that coordinates reproduction timing or specialisation. For example, in individuals created by fraternal transitions, this information integration and collective action controls reproductive division of labour (i.e., which particles get to be germ). In individuals created by egalitarian transitions, it is directed at the control of reproductive centralisation and synchronisation (i.e., the timing of particle reproduction). In either case, we can see that any susceptibility to control over particle reproduction runs counter to the fitness interests of the particle – but can confer synergistic benefits to the particle via the collective character of reproductive complementarity or coordination. Whilst reproductive control of this kind can oppose the short-term fitness interests of the individual, we hypothesise that it cannot necessarily be undone by subsequent selection because of the non-decomposable nature of the control function.[5] Thus, when information integration and collective action is directed at the control and coordination of reproductive plasticity this constitutes a new evolutionary unit. And because individual selection cannot undo this relationship, selection at the higher level can act in opposition to individual selection.

If true, how would this hypothesis inform experimental work or further theoretical development? The main impact of this hypothesis is that it makes specific predictions about the conditions for ETIs to occur that are testable either in further modelling or empirical experimentation.

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[5] This has a natural analogue in machine learning terms. When we train a neural network to represent a given function it is advisable to start from a network that is close to neutral – e.g., with small symmetric weights. If, in contrast, we train a deep network to represent a non-linearly separable function, then try to retrain from there to a new function, the learning process can become irretrievably stuck, unable to learn the second function even though the network architecture is capable of representing it.

Under What Conditions Can These Interaction Structures Evolve?

This hypothesis (H1) makes specific predictions about the conditions required for an ETI to occur and what would be required to build a working mechanistic model of a transition in individuality. Rather than a framework that depends on new genetic or selective structures that arise fully-formed, it suggests an approach where the ETIs can be smoothly integrated with more ordinary coevolutionary and social dynamics, and explains why ordinary evolutionary change, driven by selection from below, can result in transitions that later become qualitatively distinct.

H1 predicts that the difference between "ordinary coevolution" and ETIs depends on the particular nature of these relationships. If they have the effect of enacting a decomposable (linearly separable) function, particle character will be predictive of particle fitness, and this will not involve collective action, and will not constitute an ETI. However, if such a relationship becomes more non-linear over evolutionary time, it may become a non-linearly separable function. When this occurs collective character, and not particle character, will be predictive of particle fitness and an ETI has occurred.

Moreover, the difference between the kind of relationships that can constitute non-linearly separable functions and those that cannot is specific but not complicated – it just requires some depth. They cannot be represented by anything equivalent to a single layer Perceptron. Such networks do not need to be organised in neat layers as they often are in artificial neural networks such as the multilayer Perceptron – they could be messy. But they cannot be entirely shallow or have only symmetric interactions (**Box 3**).

So, how does this structure evolve? Under what conditions do deep interaction structures, computing non-linearly separable functions, evolve without presupposing the higher-level evolutionary unit we want to explain? Existing work shows several of the necessary elements (but not all in one model).

- When evolution acts on heritable variation in characters affecting the interactions between units, the effect is equivalent to connectionist models of learning ("System-Level Adaptation Without System-Level Selection: Bottom-Up Adaptation"). But, as yet, these are shallow models not deep.
- When heritable variation permits the evolution of asymmetric interaction structures, conditions exist where deep interaction structures can evolve (Nash et al., 2021). The hierarchical modularity that results mirrors the modularity of the selective environment and can consequently increase evolvability in rugged fitness landscapes. This occurs under short-term selection only, without selection for such long-term evolutionary benefits conferred by these structures. But, as yet, these models assume system-level selection.
- When individuals are given the ability to evolve symbiotic partnerships that create new reproductive units, we find that there are conditions where this permits the evolution of specific higher-level units. These units mirror the structure of the evolutionary game they are playing, and enable the discovery of high-fitness collectives that cannot be found under single-level selection (Watson et al., 2011d). These specific partnerships evolve under short-term and individual-level selection, without selection for these long-term collective benefits. But, as yet, these models assume the possibility of discrete symbiotic relationships (enacting new reproductive units) rather than collective phenotypes that develop through the signalling and plastic responses of the component particles.
- Unsupervised learning principles, acting in a decentralised manner, without system-level reward feedback or selection, demonstrate the capability to induce interaction structures that facilitate collective action and higher-level adaptation that cannot be achieved with individual action (Watson et al., 2011b). Notably this requires learned interactions to be used in a feed-forward (deep) manner rather than a symmetric recurrent manner. But, as yet, these are neural learning models not evolutionary models.

Thus, several components relevant to H1 have been demonstrated but not the whole picture in one model; we have evolutionary connectionism (in shallow models), the evolution of deep interaction structures (under system-level selection), the evolution of new selective units effective in scaling-up selection (without a developmental model), and deep models that provide collective action (in neural models). From the different components we already have, and building on H1, we hypothesise that these relationships between evolutionary individuality and deep learning models are not merely a descriptive analogy (Czégel et al., 2018, 2019) but a functional equivalence that also predicts the conditions under which bottom-up natural selection can cause these structures to evolve. Hence,

> *H2: The conditions necessary for the induction of deep models, familiar in connectionist models of learning and cognition, are predictive of the conditions necessary for an ETI to occur.*

What are these conditions?

In addition to a basic learning mechanism,[6] any learning system requires: A suitable model space (capable of representing the structure in the problem domain); A representative set of samples to learn from; And a suitable inductive bias (e.g., a parsimony pressure or other regularisation term). We address why each of these is needed in learning systems and how each of these corresponds to conditions for the evolution of transitions in individuality.

(1) A Model Space Capable of Representing the Structure in the Domain

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[6] The equivalence between learning and evolution shows that random variation and selection can provide a suitable learning mechanism (to adjust model parameters). This includes connectionist models of cognition and learning, and also deep models (Such et al., 2017; Brun-Usan et al., 2020; Nash et al., 2021) *Back-propagation*, the standard learning algorithm for the induction of deep models, is not required and a simple variation and selection process is sufficient (albeit less efficient) (Such et al., 2017).

If we want to learn correlations between system variables, for example, we must use a model space capable of representing correlations. In neural models, this just means that learning occurs by modifying weighted connections; We cannot learn anything interesting by altering the outputs (or the input-output function) of individual neurons as if they were a bag of *independent* computational units. Associative learning occurs by altering the organisation of connections in a network, not by altering the independent features of individual neural units. In learning systems this is an obvious point – but this lies in contrast to common evolutionary models, treating particles as though they are inert, and higher-selective "units" as though they are merely containers. Individuals must be modelled as non-trivial computational systems. This makes an intimate bond between organismic individuality, evolutionary individuality and cognition.

In evolutionary terms, this means that there must be heritable variation in the relationships between units – not just the independent (i.e., non-context-sensitive) features of individual particles. This means that particles must be plastic, sensitive to one-another's phenotypes, and selection must act on the details of these signal-response connections (in whatever substrate they are implemented). Then if we want to represent non-linearly separable functions, we must use a model space that can represent these higher-order functions (e.g., a structure with some depth). In evolutionary terms, this means that a shallow network architecture with symmetric interactions (Watson et al., 2014; Power et al., 2015) (e.g., a↔b, b↔c, and c↔a) is insufficient. There must be some depth to how particles interact – with some units differentiating before others (e.g., a→b, b→c, and a→c), which then have the opportunity to coordinate the behaviour of multiple downstream units (a.k.a. development) (**Figure 2C**).

(2) A Representative Experience (Samples or Training Data)

It is not possible to fit the parameters of a correlation model, let alone a deep model, from a single data sample. If we simply present a single training example and allow a Hebbian learning mechanism to alter connections, the model just learns that one pattern and canalises all the relationships between all the variables (Watson et al., 2011a,c, 2014; Power et al., 2015). To learn structural relationships, i.e., that some variables are correlated and some are not, requires a training *set* – a distribution of training samples.

In evolutionary terms, if the interactions between units are modified by natural selection after it reaches a particular attractor state, this is analogous to the presentation of a single training sample. So, if this occurs only once then relationships fitting to correlations cannot evolve. If, in contrast, the phenotypic state of units is repeatedly shocked or reset to random configurations and each time allowed to play-out to a different attractor (whilst natural selection slowly changes the relationships between them), this is analogous to learning over a set of representative training samples. This causes the future system dynamics (modified by these learned relationships) to change in a particular way. Specifically, the evolved relationships enlarge the basin of attraction for configurations that have been visited in the past (meaning that individual selection takes the system to this configuration more often in future, from arbitrary starting conditions), and crucially, also enlarges the basin of attraction for other novel configurations with especially high total utility. In the limit, as positive feedback between the states that are visited and the states that are learned builds up, the system tends to converge on only one attractor and this tends to have much higher utility than average ("System-Level Adaptation Without System-Level Selection: Bottom-Up Adaptation") (Kounios et al., 2016; Power, 2019). This is possible because the distributed associative model that is learned is not just a memory of past visited states, but a *generalised* model.

(3) A Suitable Inductive Bias

Generalisation is intrinsic to learning ("System-Level Organisation Without System-Level Reinforcement"). Of all the models that could represent the training data equally well, some will generalise differently from others, i.e., they respond differently to novel inputs. Indeed, the training set says nothing about how to respond to novel points. So, over the set of all conceivable models, it cannot be said that there are more models that categorise a novel input one way than there are that categorise it another – even if we limit this to models that agree equally well with the training set. Accordingly, the conceptual notion of "all possible models that agree with the training data" does not, in fact, afford any generalisation. Generalisation thus requires an *inductive bias*. Inductive bias describes the difference between all models that agree with the training data and the actual model delivered by the learning algorithm. Although in many contexts *bias* seems like something that should be avoided (Uller et al., 2018), in learning systems it is not – the aim is not to get rid of inductive bias, but to use an appropriate bias, that generalises well.

Accepting the idea that inductive bias is necessary for learning, the notion of a suitable inductive bias that generalises well may still seem like a cheat - a place to hide privileged knowledge that makes the system "know the right answer" despite the lack of information in the training set. It may seem like all the interesting work of a learning system is being done by this somewhat magical assumption. This is not the case. Even if we assume an appropriate inductive bias, the learning mechanism still needs to fit the model (given this bias) to the training data, and the generalisations obtained are a product of this past experience as well as the inductive bias. In fact, the form of the inductive bias can be very weak and general. For example, a bias that prefers simple models over complex models, as per Occam's razor, a.k.a. a parsimony pressure, is an extremely simple and effective inductive bias in almost all practical learning domains. In modelling terms, this can be as simple as preferring models with less connections to models that do the same thing with more connections. In biological terms, there are many reasons that simple models may evolve more readily than complex ones that do the same job. This may arise by virtue of starting from mechanisms that constitute empty or null models and adding complexity incrementally, or through subsidiary selective pressures for material efficiency, or speed, or robustness to perturbation or damage. Whatever the reason, our hypothesis predicts that this is a necessary condition for the biological networks to learn.

Here there is an important overlap between the model space of a learning system and the inductive bias of a learning system. For example, searching in the space of single-layer networks is a different inductive bias from searching in the space of multi-layer or deep networks, even if each space is explored uniformly (with respect to their own parameters). In evolutionary terms, this means that different assumptions about the nature of interactions (whether there is heritable variation that allows for symmetric or recurrent interactions, or asymmetric, feed-forward or deep interactions, etc.) will alter whether it is possible or probable to evolve non-linearly separable functions in response to the selective conditions experienced. The previous work in evolving hierarchical gene-regulatory structure shows that we do not need to assume or force interactions to be deep (Nash et al., 2021), but it also predicts that we must allow for this possibility and suggests that a strong parsimony pressure may be important in evolving such models (Clune et al., 2013; Mengistu et al., 2016; Kouvaris et al., 2017).

Learning and Evolving Deep Structures

So, what are the particular necessary and sufficient conditions for the induction of deep models in learning systems? Actually, machine learning systems usually have their topological depth prescribed by *a priori* design decisions before learning begins – systems might use a single-layer network or a multi-layer network, but whichever is used is decided at the outset and does not change during learning time [otherwise we are in the advanced machine learning topic of *topology search* (Stanley and Miikkulainen, 2002)]. However, some simple observations are useful. Shallow architectures cannot represent deep (non-linearly separable) functions but deep architectures can represent shallow (linearly separable) functions, so deep architectures are more general. And since a deep architecture can represent linearly separable functions as well as non-linearly separable functions, the depth of the function they compute can be variable even if the topological depth of the network architecture is fixed. Moreover, this is the usual progression in learning systems – by initialising a network to weights with small uniformly random values it does not, at the outset, represent a non-linearly separable function. But over learning time, it is not difficult to alter weights incrementally such that they eventually come to represent a non-linearly separable function (Brun-Usan et al., 2020). Given the possibility of moving in a suitably general model space, incremental learning algorithms are sufficient to learn such functions.

It is notable that there are learning algorithms for the single-layer Perceptron that are guaranteed to converge on any target (linearly separable) function, but for learning models capable of representing non-linearly separable functions there are no such guarantees. Back-propagation, the standard learning algorithm for deep networks, often works well in practice but does not have such guarantees. The reason is interesting. It is because the effect on the output caused by changing an input (or a weight from an input), can change sign depending on the context of other inputs. Put differently, the way that changing inputs "shows through" to the outputs is not consistent depending on the context of other inputs. In other words, the same property that makes them an interesting class of functions (for machine learning and ETIs) also makes them difficult to learn. A different way to understand this problem is that the representation learned in the hidden nodes is under-determined by the input-output relationship.[7] The learning process must break symmetry (arbitrarily) to identify a self-consistent internal representation. This is not particularly difficult (at least in functions over a small number of inputs), but the under-determination issues indicate the disconnect between selection on the outputs (collective phenotypes) and selection on the relationships between the parts therein (i.e., on the signals that turn societies into individuals). Our hypothesis H2 makes the prediction that evolving interactions that represent non-linearly separable functions, as required for ETIs, will be similarly sensitive to issues of non-guaranteed convergence and symmetry breaking. Indeed, we suggest that this is exactly why the conditions for evolving ETIs have been elusive thus far and difficult or impossible to characterise in conventional (additive) models of selection or social games. Nonetheless, we predict that deep interaction structures necessary for ETIs can evolve given the conditions identified above (and briefly summarised below).

LIMITATIONS AND CONCLUSION

The topic of the evolutionary transitions in individuality has many facets, and at present, accommodates many different opinions about what is important and how they might occur. This manuscript has been a limited discussion, positioning a particular research approach and point of view within the issues of the ETIs. This is just one attempt to try to make sense of many complex issues. Some of the limitations of our approach include the following.

- The existing models of evolutionary connectionism make a strong connection between correlation learning and evolution of relational traits, and the analysis developed here shows that such traits are critical to accessing heritable fitness differences at the collective level. The need to allow for the evolution of asymmetric interactions in order (for proto-developmental dynamics) to calculate non-linearly separable functions is also well-known. However, we have not yet put these features together in a unified model.
- As yet, we have not provided a mathematical analysis that explicitly links together non-decomposable collective characters, the response to selection at the collective level, and selection on the parameters of plasticity as an individuating mechanism that increases the heritability of these collective characters. We imagine that the direction of selection on the parameters of plasticity may be equivalent to gradients in the objective function of a correlation learning system applied to a non-linearly separable function.

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[7] Even in the trivial example of learning A XOR B, the internal representation could be ***h1*** = AND(A,NOT(B)) and ***h2*** = AND(NOT(A),B), as described in **Box 3**, or it could be the other way around, i.e., ***h1*** = AND(NOT(A),B) and ***h2*** = AND(A,NOT(B)). Either works just as well, and other decompositions are also suitable, neither construction is more right than the other, thus symmetry breaking is required to arrive at an internally consistent representation of the function.

- Other individuating mechanisms, such as mutual policing strategies, and population structuring traits, such as dispersal radii or the severity of a population bottleneck, have not been integrated into this framework. Some of our reasoning suggests that particle plasticity is the only way to remove fitness differences at the particle level whilst creating fitness differences at the collective level, but these other mechanisms and issues are clearly fundamental to many ETIs and the interaction of plasticity with these issues is currently unclear.
- Since the evolution of adaptive organisation via connectionist principles does not require that the system is already a unit of selection, there are also potentially interesting things to say about cognition, learning and individuality in systems that are not evolutionary units such as ecological communities, social systems and the biosphere.
- The relationship between non-decomposable collective characters enacted through the *phenotypic* plasticity of particles, and non-decomposable collective reproduction enacted through the *reproductive* plasticity of particles, remains unclear. If the phenotypes we are interested in have fitness consequences, the difference between regulating phenotypes and regulating reproduction may be one of degree not kind.
- At present, our approach subsumes both egalitarian and fraternal transitions under the more general concept of reproductive regulation (namely, reproductive synchronisation and reproductive specialisation, respectively). It is notable that there are two categorically different types of non-linearly separable functions (XOR and IFF) which correspond to favouring differentiation and favouring sameness. This might be connected but is not yet developed.
- Here we have mostly developed notions of information integration, and the types of interactions required to calculate non-decomposable functions, but we have not talked much about the other key feature of organismic individuality, namely collective action (except that the consequence of collective phenotypes must ultimately be applied to collective reproduction). Our computational models of deep optimisation suggest that the ability to rescale movements in phenotype space through collective action is critical to rescaling evolutionary optimisation.
- The conceptual framework presented here depends on a separation of timescales between fast variables (game strategies, selection on first-order phenotypes) and slow variables (game pay-offs, selection on second-order plasticity parameters). These correspond to the relatively fast dynamics of cognition (neural activations) and the relatively slow dynamics of learning (changes to synaptic strengths). In some biological contexts, this separation of timescales may not be clear and the consequences of this needs investigating.

Nonetheless, we have laid out a specific set of hypotheses and predictions which we hope will prove illuminating despite these limitations. We have argued that the interaction structures necessary for organismic individuality are intimately related to those required for evolutionary individuality and non-decomposable cognitive functions. Specifically, when organismic processes of basal cognition compute a collective phenotype that is a non-linearly separable function of the embryonic particle states, and this “basal decision” is applied to the control and coordination of particle reproduction, this constitutes a new evolutionary unit. This leads to the hypothesis that the conditions for deep model induction are predictive of the conditions for a transition in individuality to evolve. The potential value of these hypotheses is the specific predictions they make about the conditions for ETIs to occur. These predictions are specific enough that they are testable in further modelling or empirical experimentation. Namely, ETIs require:

- Heritable variation in the relationships between units (requiring particle plasticity and signalling) that coordinates particle functions and reproduction.
- The ability to represent asymmetric interactions structures between units necessary for deep structure (that can represent non-linearly separable functions).
- Selective conditions that are subject to repeated shocks or perturbations.
- A sufficiently strong parsimony pressure favouring simple systems.

Notably, these predictions concern features that are quite different from those commonly addressed in ETI research. For example, although measuring genetic assortment, the severity of a population bottleneck or reproductive division of labour might all be relevant to ETIs (Godfrey-Smith, 2009), they are not in themselves sufficient nor do they identify predictions about the conditions under which they will evolve. The emphasis of our hypotheses is on a unification of organismic individuality, evolutionary individuality and the principles of distributed learning – leading to a cognitive theory of individuality. This connectionist framework focusses not on changes to the frequency of units (Darwinian fitness), at one scale or another, but on the organisation of relationships between units and the conditions under which this organisation constitutes something more than the sum of the parts in a formal sense. This cognitive framework of individuality, we believe, will provide directions for future theoretical development and experimentation that begin to overcome the inadequacies of previous theoretical approaches.

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

ML and CB revised and refined the text and presented the concepts. RW, ML, and CB conceived the project together, contributed to the article, and approved the submitted version.

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The Evolution of Microbial Facilitation: Sociogenesis, Symbiogenesis, and Transition in Individuality

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Metabolic cooperation is widespread, and it seems to be a ubiquitous and easily evolvable interaction in the microbial domain. Mutual metabolic cooperation, like syntrophy, is thought to have a crucial role in stabilizing interactions and communities, for example biofilms. Furthermore, cooperation is expected to feed back positively to the community under higher-level selection. In certain cases, cooperation can lead to a transition in individuality, when freely reproducing, unrelated entities (genes, microbes, etc.) irreversibly integrate to form a new evolutionary unit. The textbook example is endosymbiosis, prevalent among eukaryotes but virtually lacking among prokaryotes. Concerning the ubiquity of syntrophic microbial communities, it is intriguing why evolution has not lead to more transitions in individuality in the microbial domain. We set out to distinguish syntrophy-specific aspects of major transitions, to investigate why a transition in individuality within a syntrophic pair or community is so rare. We review the field of metabolic communities to identify potential evolutionary trajectories that may lead to a transition. Community properties, like joint metabolic capacity, functional profile, guild composition, assembly and interaction patterns are important concepts that may not only persist stably but according to thought-provoking theories, may provide the heritable information at a higher level of selection. We explore these ideas, relating to concepts of multilevel selection and of informational replication, to assess their relevance in the debate whether microbial communities may inherit community-level information or not.

Keywords: cross-feeding, syntrophy, endosymbiosis, social evolution theory, origin of mitochondria, cooperation, public goods, major evolutionary transitions

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INTRODUCTION

Metabolic-mediated interactions and cooperation are both extremely common and widespread among microbes. However, they do not necessarily and always come hand in hand. A metabolic interaction may not be cooperative (e.g., inhibition (Netzker et al., 2020)) and cooperation may not be metabolite-mediated (e.g., host providing services like transportation to non-motile partners (Fröstl and Overmann, 1998; Bronstein, 2015)). Unsurprisingly, metabolite-mediated cooperation dominates microbial interactions, especially among prokaryotes, and in communities such as biofilms. While such complex, multi-species microbial communities are prevalent in nature, dripping with leaked metabolites and extensive cooperation, they rarely (if ever) show higher level organization.

Chemical products (nutrients, amino acids (Mee et al., 2014), siderophores (Cordero et al., 2012), enzymes (Gore et al., 2009), antibiotic degrading factors (Yurtsev et al., 2013), electrons (Stams et al., 2006), signal molecules (Antonova and Hammer, 2011), etc.) are secreted actively or leaked inadvertently by microbes into the intercellular medium, often serving the benefit of the producer and other community members as publicly available goods (West et al., 2007b; Cohen et al., 2012; Kallus et al., 2017; D'Souza et al., 2018; Smith and Schuster, 2019; Evans et al., 2020; Fritts et al., 2021) both in natural and artificial environments (Shou et al., 2007; Cavaliere et al., 2017; McCarty and Ledesma-Amaro, 2019). Public goods, however, generate conflicts between microbes (Tarnita, 2017). Those exploiting the goods increase their benefit, usually at the expense of producers, that may lead to ecological or evolutionary instability (West et al., 2007b). Nevertheless, metabolically cooperating pairwise symbioses and complex communities are both widespread and stable in the microbial world (Pande and Kost, 2017).

A *biological individual* can stably reproduce because of its replicating genes and epigenetic development. Individuals are of prime importance of biology, but are not the sole subjects of selection and evolution. Traditionally, it was believed that selection happens mostly at the lower level (those of genes) and becomes rare at higher levels (individuals, populations, species) (Lewontin, 1970). However, according to the general approach ((Hull, 1980; Dawkins, 1982; Maynard Smith, 1987), etc.), the same selection process acts on *any* entity that can multiply, inherit properties and, occasionally, variations (called *units of evolution* (Maynard Smith, 1987)), be those naked genes, cells, transient groups or populations. Consequently, *selection may act at multiple levels* concurrently, of genes, cells, populations (Okasha, 2005). And it is only a quantitative matter how much information at a given level can be stably inherited from generation to generation. Transition theory (Maynard Smith and Szathmáry, 1995; Szathmáry and Maynard Smith, 1995) posits that units can traverse these levels. For example, independent cells may form transient associations to increase their overall success, and, ultimately, waive their individual replication for the greater good of the group. When such entities are irreversibly coupled so that none can replicate without the other, a bona fide new unit of evolution emerges and a transition in individuality is made (Estrela et al., 2016).

According to transitions theory, cooperation may be selected for at a higher level and could stabilize higher level evolutionary units, integrating individually reproducing cells (Szathmáry, 2015). Syntrophic microbial partnerships may strengthen to form obligate symbiosis. For example, most archaea live in obligate dependence of their syntrophic partners (Pande and Kost, 2017). But the interesting question is: can metabolic cooperation itself lead to a major transition in individuality (Maynard Smith and Szathmáry, 1995; Szathmáry and Maynard Smith, 1995)? In such a transition, previously free-living individuals evolve toward strong dependence so that they can only reproduce as part of an integrated collective (Michod and Nedelcu, 2003; West et al., 2015). Transitions theory is predominantly interested in how conflicting interests and cooperation may yield multiple levels of selection (Okasha, 2005) and collectives as new evolutionary units. For example, how microbes overcome their selfish interests to form a mutually dependent cooperative collective?

While evolution toward a transition increases the degree of symbiotic organismality (i.e., integration), the transition, ultimately, is characterized by strict vertical transmission and mutual dependence of partners (West et al., 2015; Estrela et al., 2016). The threshold separating these two phases is not well defined, and we usually are only aware of cases that have already concluded. Before such a transition, interspecies interactions are known to be context-dependent, shifting freely between parasitic, commensal or mutualistic (Bronstein, 1994; Chamberlain et al., 2014; Chomicki et al., 2020) as partners adapt to each other and to changes of the environment. The outcome depends on the net costs and benefits of association (Estrela et al., 2016). It is thus not clear how and why a metabolic partnership may stabilize under higher-level selection. We set out to understand whether microbial communities possess (have ever possessed or may acquire) the necessary properties to qualify as units of selection. We review the evolutionary trajectories of metabolically coupled (cross-feeding) microbial communities where selection may favor reciprocal cooperation (mutualism) and possibly entail dependence and integration. We ask whether and how such communities may become collectives, i.e., units of evolution and make a transition in individuality. We explore these trajectories from the general points of view of replicator theory and multilevel selection. This may help identifying microbial interactions that entail multilevel selection and may help recognizing how far particular communities have advanced on the path toward an irreversible transition in individuality, and ultimately provide predictions of evolutionary transitions in microbial systems.

COOPERATION AND MUTUALISM

Cooperation, in general, refers to an ecological interaction between any two individuals, conspecifics or not, where at least one enjoys a benefit (often *cooperation* is reserved for intra-species interactions, and *mutualism* for inter-species interactions). Cooperation, formally described, is a higher-order interaction among replicating entities (Szathmáry, 2013), for example species. It is an interaction where one species exerts a positive effect on the reproduction rate of another species. In other words, species A aids the reproduction of species B. In chemistry language, this is called cross-catalysis, and means that the overall growth of species A depends on the density of species B. This definition so far does not assume anything about costs payed and benefits received by A, or that there is any reward or reciprocation from B. Cooperation is an "action that is beneficial to the recipient regardless of its effect on the donor" (Sachs et al., 2004), that is, a cooperative act may or may not be costly (see later). Nevertheless, in the evolutionary context, paying to help other organisms will be selected against, unless there is also benefit for the cooperator, perhaps averaged over a longer timespan, multiple contexts, or kin (West et al., 2006). This condition ensures that trophic interactions, such as microbial predation (where the prey also benefits the predator in a density-dependent manner), are not categorized as cooperation.

If the benefit is reciprocated, the interaction is reciprocal cooperation, or mutualism (Bronstein, 2015). Cooperation can be nutrient mediated (e.g., exchanging metabolic components) or service type (e.g., transportational or protective) (Bronstein, 2015). Such reciprocity may not be apparent when looking at, e.g., metabolic interactions, as it often looks like exploitation (Sørensen et al., 2019). This is because benefits may realize in different forms and at different timescales, from nutritional and transportation to protection benefit [for more details see Zachar and Boza (2020)]. Symbiosis is when partners of different species live in a physical contact or integration in most or all of their life cycles for a prolonged time allowing evolutionary adaptations to take place. Symbiosis is not necessarily mediated by metabolites, nor is necessarily mutually beneficial (Bronstein, 2015). A special case of symbiosis, involving metabolic interactions is called cross-feeding and syntrophy.

HOW MICROBES COOPERATE: SYNTROPHY AND BIOFILMS

Most of cooperation in the microbial world is dominantly mediated by metabolites (Fritts et al., 2021). Metabolite exchange by itself, however, does not induce cooperation: a metabolic interaction may or may not provide direct or indirect benefit for one or more party, producer included. If there is benefit for at least one partner, the metabolic interaction entails an (often implicit) catalytic aid (**Figure 1D**). Moreover, the benefit for the recipient depends on the product produced by the donor, which can be safely assumed to correlate with the density of the donor.

Cross-feeding (or syntrophy) is a special case of microbial metabolic cooperation, relying on (accidentally or intentionally) externalized (by-) products. Historically, cross-feeding referred to microbial interactions involving transfer of molecules entailing enhanced growth of participant species (D'Souza et al., 2018). Often, definitions focus on the transfer of compounds (Morris et al., 2013), on enhanced growth in the presence of partners (Fritts et al., 2021), on mutualistic nutrient exchange (Searcy, 2002), on obligate dependence, without which none can grow (Libby et al., 2019), or on the possibility of enabling new resources and niches (Schink, 2002; Stams and Plugge, 2009; Libby et al., 2019). Others simply define syntrophy phenomenologically as an interaction involving leaked products that increase the carrying capacity (or growth) of one or more species[1] (Jimenez and Scheuring, 2021). There is a rather diverse range of definitions in the literature (see D'Souza et al., 2018; Smith et al., 2019), rendering cross-feeding and syntrophy umbrella terms. Although different types of metabolic interactions rely on different mechanisms that imply different dynamics (see later), the literature in general fails to properly formalize, blurring fundamental differences between them. For the sake of simplicity, we will use syntrophy to denote a metabolite-mediated interaction that is beneficial at least to one party.

Syntrophy can form between phenotypically different or spatially displaced individuals of the same species (Liu et al., 2015), or between different species (Shou et al., 2007; Hillesland and Stahl, 2010; Goldford et al., 2018). The significance of syntrophy in ecosystem functioning and evolution is clear for three different reasons. First, syntrophy seems to be widespread, ubiquitous, and easily evolvable. As it turns out, microbes, especially archaea (Castelle et al., 2015), depend heavily on products of other prokaryotes due to syntrophic interactions, being potentially responsible for the unculturability of archaea (Pande and Kost, 2017) and bacteria (Staley and Konopka, 1985). The ubiquity of microbial auxotrophies (D'Souza et al., 2018) and metabolic cooperation indicate that it is easy to encounter complementary metabolisms among microbes, even without prior co-evolutionary history. Second, metabolic community, and especially biofilm, design and stability became one of the leading research areas of artificial microbiology and biotechnology (Hays et al., 2015; Libby et al., 2019; McCarty and Ledesma-Amaro, 2019). Third, our increasing understanding of the frequent metabolic dependencies of archaea on microbial partners (Pande and Kost, 2017) and the fact that there is extensive gene transfer between microbial community members (Gogarten et al., 2002; Madsen et al., 2012) have shifted mitochondrial origin hypotheses from early phagocytosis to metabolic syntrophy (Zachar and Szathmáry, 2017; López-García and Moreira, 2020). According to these, mitochondrial endosymbiosis has emerged from mutually beneficial syntrophy of an archaeon and a bacterium that may have evolved as part of a community rather than as separately living organisms (Martin et al., 2015; Spang et al., 2019; Imachi et al., 2020; López-García and Moreira, 2020). As such, syntrophy may have been the starting point of one of the most intriguing major evolutionary transitions, the emergence of eukaryotes.

According to Dennis Searcy, there are two major patterns of syntrophy, flow-through and recycle, and only the latter is capable of accumulating nutrients (Searcy, 2002). They depend on different mechanisms that likely entail different dynamics.

Flow-through or **waste-removal**: The product can accumulate and self-inhibit the producer, while the partner cannot grow without the product. Removing the waste helps both the receiver and the producer to grow. The benefit depends on the rates of production and removal. For example, H_2 production in methanogenic communities and by endosymbionts (Fenchel and Finlay, 1991; Embley and Finlay, 1994; Lengeler et al., 1999;

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[1] According to cooperation theory, increasing population density of the partner species increases the relative fitness of receiver species (Dobay, 2014). In microbial context, this amounts to the assumption that products realize the cooperative benefit, and the cooperative efficiency of a product depends on its concentration. Since product concentration usually depends on the density of producers, ultimately the benefit of cooperation depends on the density (and production rate) of producers, as was assumed by, e.g. (Jimenez and Scheuring, 2021). The benefit of a species is thus ultimately realized in the increased growth rate or carrying capacity in such a model.

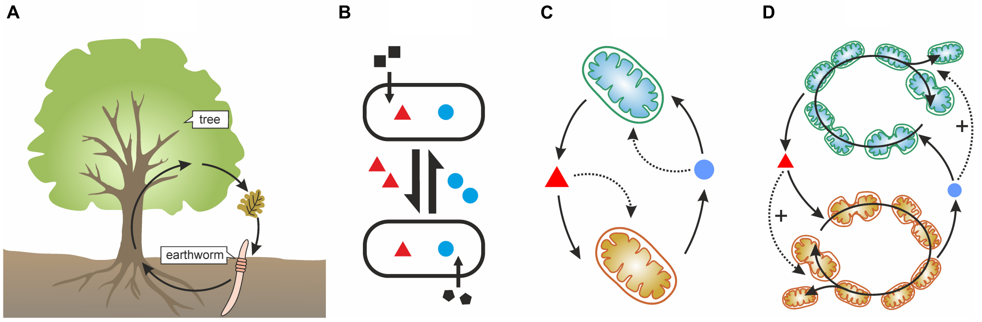

FIGURE 1 | Different representations of ecological interactions. **(A)** An ecological hypercycle between two organisms [from Maynard Smith and Szathmáry (1995), p.58]. This example is deliberately confusing: without further specification, arrows could represent purely trophic interactions, cooperation, or both. It is also not immediately apparent if there is any auto or cross-catalysis going on. Clearly, a better representation is required to give a full account of the relevant interactions. **(B)** Bidirectional cooperative cross-feeding with reciprocal exchange of a costly metabolite (red triangle, blue disk) that benefits both partners, as depicted in D'Souza et al. (2018). Note, that neither the cost, nor the benefit (cooperative act) is obvious without additional information. As a matter of fact, arrows could simply indicate the transformation of matter without any cooperative aid. **(C)** A more accurate representation of a syntrophic interaction: normal arrows indicate production and consumption, dotted arrows indicate cooperative benefit (catalytic aid). **(D)** A more precise representation of syntrophy with reproduction made explicit: benefit is only cooperative if it enhances the growth of the receiver, that is, it feeds back positively on its reproduction.

Fenchel, 2006; Nowack and Melkonian, 2010; Madigan et al., 2014), or proteobacterial ectosymbionts improving growth of aerobic protists by removing toxic photosynthesis waste (Hünken et al., 2008). A special case is the situation where "organisms combine their metabolic capabilities to catabolize a substrate that cannot be catabolized by either one of them alone" (Stams and Plugge, 2009). For example, methane formation and oxidation by syntrophic communities of archaea and bacteria where reducing equivalents are transferred in an interspecies electron transfer (Stams and Plugge, 2009). In obligately syntrophic communities, for the degradation of specific organic compounds and growth, both the archaeal and bacterial partners are essential, no one can degrade it alone. Regarding the origin of mitochondria, hydrogen hypothesis (Sousa et al., 2016) and organic acid syntrophy (John and Whatley, 1975; Searcy et al., 1978; Margulis, 1981) fits here.

Nutrient recycling: If a resource is limited in the environment, neither species can grow unless one can recycle it back to its initial state becoming available for consumption again. Species therefore depend on each other. For example, sulfur cycling between reducer heterotroph *Sulfurospirillum* and oxidizer photosynthetic *Chlorobium* (Wolfe and Pfennig, 1977), anaerobic ciliate *Strombidium purpureum* and endosymbiotic PNSB (Fenchel and Bernard, 1993a,b). In the mitochondriogenesis context, sulfide hypothesis (Searcy, 1992, 2014) and syntrophy hypothesis (López-García and Moreira, 2020) belong here. This type is especially important, if the cycled product is required only in catalytic amounts (such as enzymes) and is quickly regenerated. The benefit in such an interaction depends more on the rate of regeneration than on the rate of production, the former being presumably faster.

We must emphasize an important point here. Many interpretations and illustrations of the syntrophy literature is insufficient or downright misleading, suggesting that metabolite exchange automatically entails cooperation. However, this is not necessarily true. The conversion of metabolites *and* the cooperative benefit of these interactions cannot be represented with a single type of arrow in a simple interaction graph (**Figure 1B**). Both metabolite transformation *and* catalytic interactions (benefit) must be explicitly and differently represented to account for microbial cooperation (**Figures 1C,D**). The classical illustration of a tree and a worm forming a cooperative cycle of **Figure 1A** is misleading as it simultaneously depicts trophic and cooperative interactions between partners, without differentiating between them explicitly.

Cooperation, such as producing a shared metabolite, is a costly act that benefits the recipient, hence the fitness of the actors is decreased while the recipient's is increased. It is easy to see that without additional mechanisms strategies that do not produce but enjoy the benefits (free-riders or cheaters) are expected to reproduce faster and overtake the population (West et al., 2006, 2007a; Nadell et al., 2009; Tarnita, 2017; Smith and Schuster, 2019). Consequently, extracellular materials may act as public goods, generating social conflicts between microbes (Tarnita, 2017). There is a subtle difference between public (or shared (Cavaliere et al., 2017)) and common goods. Public goods are non-rivalrous (one using it does not make it less valuable for others) while common resources are rivalrous (one using it diminishes its accessibility or value for others) (Kollock, 1998; Dionisio and Gordo, 2006). For example, the extracellular biofilm matrix or extracellular enzymes can be characterized as public goods, while extracellular nutrients or leaky macromolecules as common goods. From a game-theoretic point of view, cooperators contribute to the production of the public good (or refrain overconsuming the common resource), while defectors do not contribute to public good (or overexploit the commons). As a result, defector dominance results in the degradation of the collective resource (Smith and Schuster, 2019). For example, the presence of cheater strains in *P. aeruginosa* reduced the thickness and density and the population growth of the biofilm, as such strains do not contribute in extracellular factor production (public good), but enjoy the benefits (Popat et al., 2012). Analogously, a “wasteful” strain with a high rate of ATP production displaces a “prudent” strain with a high yield of ATP production when competing for an extracellular common resource, leading to a tragedy of the commons (MacLean, 2008). For more information on the challenges posed by free-riders in syntrophic communities (endosymbioses included), the Reader is referred to Zachar and Boza (2020), especially to section “Issues of syntrophic consortia.”

The most widespread of structured microbial communities are biofilms, in which microbes from all domains establish diverse social interactions (West et al., 2007b). Biofilm formation appears to be an ancient prokaryotic feature (Hall-Stoodley et al., 2004). Biofilms can appear on any surface, from hydrothermal vents and freshwater bodies through soil and leaves to the internals of multicellular hosts. They develop by aggregation exhibiting both temporal and structural succession and differentiation (Hall-Stoodley et al., 2004). As a result, recruitment of species, differential gene expression and development of phenotypes are ordered (Stoodley et al., 2002). Biofilms always produce an extracellular matrix, secreted by all or some members, and serves as the medium for various metabolic and facilitating processes. The matrix sticks cells together, providing protection against external hazards (e.g., grazing (Seiler et al., 2017)) but also forms an internal network to facilitate water flow and the exchange of resources like public goods (Stoodley et al., 2002). In multispecies biofilms, multispecies spore formation is not expected, thus they disperse by coordinated degradation of the matrix (Davies, 2011) or by fragmentation via single cells or clusters (Stoodley et al., 2001; Rumbaugh and Sauer, 2020). This imposes a serious bottleneck resulting in new colonies being subject to a severe founder effect (see later). Due to the structure of biofilms, interactions are localized and neighborhoods are stable for a prolonged time. In biofilms, but also in other forms of microbial communities, metabolic cross-feeding (*sensu* (D’Souza et al., 2018)) and syntrophic microbial partnerships are ubiquitous and diverse (Morris et al., 2013; Pande and Kost, 2017). Since most prokaryotic species cannot be cultured without partners (Staley and Konopka, 1985; Pande and Kost, 2017; Imachi et al., 2020), metabolic complementarity is expected to be common and stable in nature. Furthermore, since syntrophy is a form of cooperation, it is expected to stabilize larger communities too.

HOW MUTUALISM MAY EVOLVE IN MICROBIAL COMMUNITIES

In general, ecosystems evolve toward increased productivity and higher metabolic efficiency, leading to decreased productivity/biomass ratio and tighter nutrient cycles (Odum, 1969; Loreau, 1998). Both within-cycle and between-cycle (in terms of material cycles) competition constitute selective forces that drive this process (Loreau, 1998). One would expect that natural selection, after sufficiently long time, eliminates inefficient types and maximizes energy fitness by optimizing the growth, reproduction, and survival rates, and the efficiency of energy production (Burger et al., 2021). There are, however, a few limiting factors and trade-offs (de Lorenzo et al., 2014; Cavaliere et al., 2017; Burger et al., 2021). First, the lack of sufficient genetic variation caused by the continual selection that reduces heritable variation and hence gradually slows down the evolution of improvements. Second, ecological compensation and limits to growth. As evolution increases efficiency considerably, such a population will start to grow faster and counteracting environmental (ecological) limitations, such as the carrying capacity or the increased appeal as a resource of a population with high biomass for predators, will become stronger impeding the initial growth advantage. Third, continual coevolution and the Red Queen mechanism. Since the overall biomass energy delivered by the net primary production is the ultimate limiting resource for life in the biosphere, there is a continual selection on all species to increase their shares (Burger et al., 2021). Due to the continuous ecological and evolutionary dynamics of species, and often also changing environmental conditions, evolving a best strategy in a certain biotic and abiotic environment can only give temporary advantage. The complex relationship between ecological interactions and coevolution of species leads to fluctuating levels of inefficiency at the species level, but higher efficiency at the ecosystem level (Burger et al., 2021). Such a scenery, however, allows for the enhancement of energy uptake by means of inter-species cooperative metabolic interactions.

Segregating conflicting or complimentary metabolic pathways into separate compartments (such as protocells and cells) and securing the increasingly efficient flow of metabolites between these compartments (i.e., syntrophy), either through a medium or through special structures (Mori et al., 2016; Fritts et al., 2021), offers a very powerful way to increase metabolic productivity (van Gestel et al., 2015; West and Cooper, 2016; Tsoi et al., 2018). Such pathway modularization, a community-level division of labor, can emerge under various conditions (de Lorenzo et al., 2014; Cooper and West, 2018), and relies on social interactions between partners.

Consequently, the emergence of metabolic division of labor is driven by trade-offs in unicellular organisms, and can be selected for in several contexts (Goel et al., 2012; Großkopf and Soyer, 2016; Stump and Klausmeier, 2016; Dragoš et al., 2018; Louca et al., 2018). For example, a trade-off between metabolic efficiency in terms of rate and yield in ATP-producing pathways is hypothesized (Pfeiffer et al., 2001; Helling, 2002; Gudelj et al., 2007), which tradeoffs materialize because of the presence of alternative pathways in ATP production and the related thermodynamic principles (Pfeiffer and Schuster, 2005). Evolutionary game theory-based analysis reveals that if such alternative pathways are available, evolution should favor the fast but inefficient energy-production strategy feeding on a shared primary resource. Once such “wasteful” strategy outcompetes others and becomes dominant, their waste product also accumulates in the medium. In theory, the ratios of wasteful strategies and strategies feeding on their waste products can reach an equilibrium (Doebeli, 2002; Pfeiffer and Bonhoeffer, 2004; Pfeiffer and Schuster, 2005), in general favoring the emergence of stable cross-feeding interactions (Hansen et al., 2007; Germerodt et al., 2016; Gudelj et al., 2016; Cavaliere et al., 2017; Stump et al., 2018).

Besides the rate-yield type of conflicts, many other forms of conflicts, trade-offs, or complementarities can serve as the driver for the spatial and chemical segregation of cellular processes leading to bacterial polymorphism and cooperation (Doebeli, 2002; Gudelj et al., 2007, 2010; de Lorenzo et al., 2014; Meijer et al., 2020). The main cellular biochemical conflicts include competition for intracellular resources (ATP, synthesis machinery, cellular space, etc.), incompatibility and inhibitory conflicts, and enzyme-specificity conflicts (Gudelj et al., 2010; Johnson et al., 2012; de Lorenzo et al., 2014). While some conflicts can be resolved intracellularly (de Lorenzo et al., 2014), inter-cellular arrangements are often more effective (Johnson et al., 2012). Principally, the power of symbiosis lies in the fact that genetic and metabolic machinery from very distantly related organisms can be brought together (Maynard Smith, 1991; O'Malley, 2015). The new unit now can be treated as a dramatic mutational change allowing for a wider range of adaptations. Such an effect may be less pronounced, but still holds for microbial syntrophy and cross-feeding interactions as well.

There is ample evidence that syntrophy and metabolic complementation indeed evolves under various conditions (D'Souza and Kost, 2016; Großkopf et al., 2016; Mori et al., 2016; Van Hoek and Merks, 2017; Kallus et al., 2017; Meijer et al., 2020), and significant metabolic synergy can be found even between randomly paired partners without co-evolutionary history but with certain metabolic deficiencies (Wintermute and Silver, 2010). For example, securing physical proximity (ecological integration) induce evolutionary processes strengthening reciprocal fitness feedbacks resulting in a stable mutual cross-feeding between autotrophs (Harcombe et al., 2018; Preussger et al., 2020), although there is also a chance that the strains break free of the initial by-product based partnership (Preussger et al., 2020).

The most commonly known mechanism driving the evolution of bacterial syntrophy is provided by the Black Queen hypothesis, stating that leaky public (or shared) goods and selection for small genome sizes drive the evolution of mutual dependency between the members of a consortium (Morris et al., 2012). The genome size is decreased as cellular economization to ensure faster growth; often referred to as the streamlining hypothesis (Giovannoni et al., 2014; Martínez-Cano et al., 2015). At the same time, genomic reduction can often be understood as a community-dependent adaptive event: the lost functionality can be compensated by other members still possessing the necessary genes and functionality that benefits the whole community via a commonly available public good (Morris et al., 2012; Martínez-Cano et al., 2015). Such an adaptive gene loss can result in a diversity of, pair-wise or networked, mutualistic interactions and could potentially trigger the evolution of more specific and intimate mutualisms (Morris et al., 2012, 2013; Estrela et al., 2015, 2016).

Determining the fitness benefits arising from such dependencies and unraveling the reversibility of such relationships is particularly crucial. In evolutionary theory, we can distinguish proximate and ultimate mutualistic dependency (De Mazancourt et al., 2005; Zug and Hammerstein, 2014; Chomicki et al., 2020). Although both can be measured by removing the partner and by comparing the performances before and after removal, the picture appears to be more complex and fuzzy. Firstly, measuring the benefits of mutualisms, either in mono- or in co-cultures, and choosing the most evident measure, such as biomass, yield, growth rate, resistance against external perturbations, etc. (including biotic or abiotic sources), and the time-horizon during which performance in monitored, remains a puzzling issue (Mitri and Foster, 2013; Chomicki et al., 2020). Secondly, there are examples in which removal of the partner results in a short-term decrease in performance, or can even be lethal, but this may be due to the shared long-term co-evolutionary history, rather than evolved interspecies cooperation (De Mazancourt et al., 2005). Such an evolved dependence may be the result of adjusting to the presence of a non-beneficial (neutral or harmful) partner from which there is no getting rid of, and after long evolutionary time, the removal of this partner can now be detrimental for the host (Jeon, 1995, 2004; Weinbauer, 2004; Fellous and Salvaudon, 2009; Mitri and Foster, 2013; Zug and Hammerstein, 2014; Weinersmith and Earley, 2016). But this does not mean that the host couldn't perform well without the non-beneficial partner, only means that the observed genotype is not adopted to the absence of it (De Mazancourt et al., 2005). Compared to such an evolved dependence, in true ultimate mutualisms real benefits are associated with the partnership and indeed none could have performed as well without the other (De Mazancourt et al., 2005; Zug and Hammerstein, 2014; Chomicki et al., 2020). Note that true mutualism can also evolve from evolved dependence, as we see instances of antagonisms evolving into mutualistic symbiosis (Fellous and Salvaudon, 2009; Sachs et al., 2011, 2013; Chomicki et al., 2020).

In a cross-feeding microbial community, the metabolic performance, and therefore the fitness of each cell depends directly on a subset of community members (those in direct interaction with the focal cell, metabolically or otherwise), and indirectly on the connected community as a whole (Khandelwal et al., 2013). Although formally the interaction network consists of pairwise interactions of cells (Faust and Raes, 2012), it can also enable higher-order interactions that may play a critical stabilizing role in complex microbiomes (Levine et al., 2017). While the interactions are mostly cooperative (West et al., 2006, 2007a), a shared metabolism can also be antagonistic (Machado et al., 2021). There exist highly cooperative communities, typically composed of members with smaller genomes and a diversity of auxotrophies. But there are also highly competitive communities with larger genomes, overlapping nutritional requirements, and higher antimicrobial activity (Machado et al., 2021). It seems that competitive communities can better resist species invasion but not nutrient shift, whereas cooperative communities are more susceptible to species invasion but resilient to nutrient changes (Machado et al., 2021).

The division of metabolic labor may trigger niche emergence or construction by opening novel, more efficient, or previously inhibited pathways enabling new metabolic phenotypes (Pearman et al., 2008; Colwell and Rangel, 2009; Großkopf et al., 2016; Ponomarova et al., 2017; Gatti et al., 2018, 2020; San Roman and Wagner, 2018; Oña et al., 2021). At the same time, division of labor results in a social interaction that have fitness consequences for both the producer and the recipient and leads to collective functionality (Crespi, 2001; West et al., 2006; Ackermann, 2015; Hays et al., 2015). For example, in the H_2-mediated syntrophic interactions in methanogens, the metabolism of the secondary degrader inhibits further growth unless the syntrophic partner consumes H_2 (Cavaliere et al., 2017), hence growth is only possible under coexistence.

It has been demonstrated that the evolved members of a syntrophic consortia are fitter than the non-evolved ancestral strain and that the consortium itself demonstrates enhanced biomass productivity, often interwoven with reduced byproduct accumulation (Bernstein et al., 2012; D'Souza and Kost, 2016; Harcombe et al., 2018; Preussger et al., 2020; Yang et al., 2020). Furthermore, coevolution of obligate mutualist bacteria can result in a faster growth and increase in productivity (Hillesland and Stahl, 2010; Summers et al., 2010). Growth advantage (increased Darwinian fitness) due to division of metabolic labor can be measured relative to prototrophic wild-type cell cultures (Pande et al., 2013; Pande and Kost, 2017). Enhanced metabolic activity is another sign of the synergistic effect of cross-feeding compared to cultures in which all reactions take place within one organism (Martínez et al., 2016). Densely connected syntrophic networks may partly suppress competition within guilds whose members would be in strong competition with each other for a common resource otherwise (Goldford et al., 2018).

Naturally, the fitness effects (i.e., costs and benefits) of metabolic interactions are often context dependent (Bronstein, 1994; Chamberlain et al., 2014; Hoek et al., 2016; Zengler and Zaramela, 2018; Chomicki et al., 2020) and may shift back and forth on the mutualism-antagonism continuum (Drew et al., 2021). Factors, such as intermediate cell densities and medium level of spatial proximity (Kim et al., 2008; Bull and Harcombe, 2009; D'Souza, 2020), presence of other parties, cooperative or exploiter types, in the community (Harcombe et al., 2016; van Tatenhove-Pel et al., 2021), as well as resource availability are all expected to affect the net benefit (Drew et al., 2021).

HOW MAJOR TRANSITIONS MAY EMERGE IN MICROBIAL COMMUNITIES

A central tenet of transition theory and multilevel selection is that cooperation of lower level units realize group-level synergies that can be selected for at a higher level, ultimately establishing a new, higher level unit of evolution, the group (Szathmáry, 2015). There are a multitude of multicellular microbial communities based on metabolite-based cooperative aid where related cells stay together after division or aggregate via signals. Staying together may lead to canonical multicellularity, simple or complex, of a single species as in, e.g., cyanobacteria or metazoa. There are several social microbial species that evolved such an aggregative multicellularity (Rainey and Rainey, 2003; West et al., 2007a; Boyle et al., 2013). For an overview of the different community types, see **Table 1**.

However, there is a great difference between a single-(social) species and a multispecies community. Social multicellularity depends on the cooperative behavior of members, potentially having different phenotypes. Metazoa represent a typical case of fraternal major transition (Queller, 2000), where sister cells waived their reproductive ability for the common good of reproducing the multicellular body, with sophisticated epigenetic inheritance methods (Jablonka and Lamb, 1989, 1995). Interestingly, no multispecies microbial community seems to have ever made this transition and evolved to inherit epigenetic information, possibly because they involve cooperation of genetically unrelated partners which would require an egalitarian transition entailing conflicts that are hard to police. Here, we focus on evolutionary routes, that can potentially lead to higher level selection in communities, further integrating partners and may (at least theoretically) open a door to a transition in individuality (see **Figures 2**, **3**). We set out to explore potential heritable information of multispecies communities.

If there is a higher level of selection, there must be some form of population structure, which can facilitate cooperation (Nowak, 2006). Since cooperators sustain the structure (community, biofilm, group), they can spread better under selection. It would be informative to approach the problem at hand from a more abstract viewpoint. One may extend the concept of reciprocal cooperation of syntrophic microbes to more than two species. Accordingly, a metabolic community is a set of cooperative species, where cooperative help is mediated by produced public goods. The aid may chain (A helps B, B helps C) and form a network, that would stabilize member species. Ultimately, but not necessarily, a cycle may form (C helps A). It is formally called a hypercycle, a theoretical proposal to explain how (prebiotic) replicators could have coexisted via cross-catalysis (Eigen, 1971). Theoretically, such a cross-catalysis between organisms can itself be autocatalytic (multiply), and would feed back positively on all of the member species and on the collective itself. If so, it can be superior compared to linear (or non-) cooperative networks, as it can grow in numbers or compensate against loss via degradation, side reactions, mutations (Maynard Smith, 1979). If adaptive traits benefiting the collective emerge and are inherited, they can be selected for at the collective's level. In the microbial domain, it means that adaptations that strengthen the group (via nonrandom recruitment, association and co-occurrence of partners) may accumulate and lead to the stabilization of the population structure. We emphasize that no hypercycle is known to exist in the microbial world. The real question is, can anything higher than species inherit changes in a real mutual microbial community? And can an evolutionary transition in individuality (West et al., 2015) happen? The answer is not trivial and is thoroughly debated.

Biofilm as Replicator

A long-standing debate of evolutionary biology is concerned with the "true" units of evolution (Williams, 1966; Wilson, 1975; Maynard Smith, 1987). A particularly interesting case is about the potential reproduction of multispecies microbial metabolic communities, namely, biofilms. According to some, biofilms are true reproducing units of evolution on their own right (Ereshefsky and Pedroso, 2012, 2015; Doolittle, 2013), while others maintain that they lack the necessary mechanisms and

TABLE 1 | Properties and examples of various types of community-forming and transitions in individuality.

Community type	*N*	Division of labor	Higher evolutionary unit (*putative*)	Examples
Single species aggregative community	1	Between different phenotypes.	Never transited completely to a new unit of evolution. *Interaction and developmental patterns may be reproduced.*	Biofilm-forming social microbes, e.g., *Pseudomonas fluorescens* (Rainey and Rainey, 2003), *Vibrio cholerae*, *Xanthomonas campestris* and *Staphylococcus aureus* (Solano et al., 2014), etc.; eukaryotic cellular slime molds (*Dictyostelium*).
Single species staying-together community	1	Between different phenotypes.	Some completely transited to higher levels (e.g., animals, fungi, plants), some not (Cyanobacteria).	Cyanobacteria, Metazoa, Fungi, higher plants (Embryophyta), brown and golden-brown algae, red algae, green algae, charales, etc.
Multispecies aggregative community	2+	Between different species and phenotypes.	*Entire community or parts of it that may form lineages.*	Multispecies microbial biofilms, communities of multicellular organisms, ecosystems, biogeochemical cycles (Fritts et al., 2021), Gaia (Stolz, 2016).
		Between different species and phenotypes.	*Interaction and developmental pattern.*	The joint metabolism, regulated assembly, developmental interactions and phenotypic functionality of multispecies biofilms may reproduce at the community level (Doolittle and Booth, 2017).
		Between different species.	Strongly coupled ecto- or endosymbiotic pair.	Many symbiont-host examples exist (parasitic, syntrophic, exploitative), but it is unknown if they have evolved as part of a community or not. Among prokaryotes, the only example is the presumed endosymbiosis of mitochondria and their hosts.
Free-living symbiotic pair	2	Between different species.	Ecto- or endosymbiotic pair.	

A new level of selection may evolve when individuals replicate together better than random and have adaptations at the group level. However, a bona fide new unit of evolution emerges only if individuals waive their independent replication to only reproduce with the group. Putative new units of evolution are in italics.

inheritance pathway to stably pass on information (Nadell et al., 2009; Clarke, 2016), e.g., changes in biofilm composition akin to a mutation in the genetic code.

Multiple arguments seem to support biofilms as units of evolution. Members exhibit coordinated activity (Davies, 2011; Lyons and Kolter, 2015), and cooperate in a reciprocal and network-like topology. They make costly investments that are beneficial for the producer and for others too (Jimenez and Scheuring, 2021). Benefits may be realized at the collective's level (e.g., extracellular matrix) (Mitri and Foster, 2013; Ren et al., 2015). There may be further biofilm-level adaptations, however, Clarke has found these not verifiable (Clarke, 2016). Lichens share many similarities to biofilms (Carr et al., 2021; Libby and Ratcliff, 2021), and they clearly maintain characteristic types, that carry on the specific mycobiont and photobiont in successive lineages, and appear to involve multiple (prokaryotic) partners (Nelsen et al., 2020; Carr et al., 2021). Lichens provide an interesting analogy to biofilms as units of selection (Libby and Ratcliff, 2021), while their development is clearly differing from the sometimes *ad hoc* assembly of microbial biofilms.

Clarke has assessed the possibility that (multispecies) biofilms (or arbitrary subparts of it) may be group-selected units of evolution (Clarke, 2016), and has convincingly argued, that they lack crucial aspects to be *bona fide* groups under selection. Biofilm cells do not differentiate terminally, and there is not reproductive division of labor (no lineage loses irreversibly its reproductive capacity). Members can often be freely exchanged with functionally equivalent species interactions not being species-specific. Component biofilm lineages do not migrate collectively to new niches (Kolenbrander et al., 2010). There is no real group structure in the biofilm on which group selection could act, only varied local interaction networks. Without a macrobiotic host, colonization does not happen via vertical transfer (as in termites), neither do they form multispecies-spores, lacking thus an essential mechanism for stable co-occurrence of species. Hence, they do not form lineages that compete and in which biofilm-level adaptations are inherited from parent to offspring biofilm. This is because multispecies biofilms may not reproduce their structure due to fragmentation (cf. founder effect, see (Brislawn et al., 2019)) but via successive assembly during which members are horizontally recruited from the local environment (Moran and Sloan, 2015; Clarke, 2016; Douglas and Werren, 2016).

This is the appropriate time to elucidate on a subtle difference, that is often confused by those claiming that anything that is not a conventional organism (bounded by skin or vesicle and reproducing as a unit) cannot be subject to selection. We point out, that multilevel selection should not be interchanged with group formation and especially with evolutionary individuality. Selection can act on collectives, given that there is some better than random chance that members reappear together from time to time. Selection can act not only on compartmentalized (bounded) replicators, but also on temporally compartmentalized (Wilson, 1975, 1979; Matsumura et al., 2016), or no compartmentation at all in the conventional sense (e.g., spatial models, like metabolically coupled replicator systems (Czárán et al., 2015)), albeit admittedly with weaker effect. Their dynamics can be described by models of multilevel selection of the first type (MLS1), compared to the group selection dynamics of real evolutionary individuals that are themselves collectives of

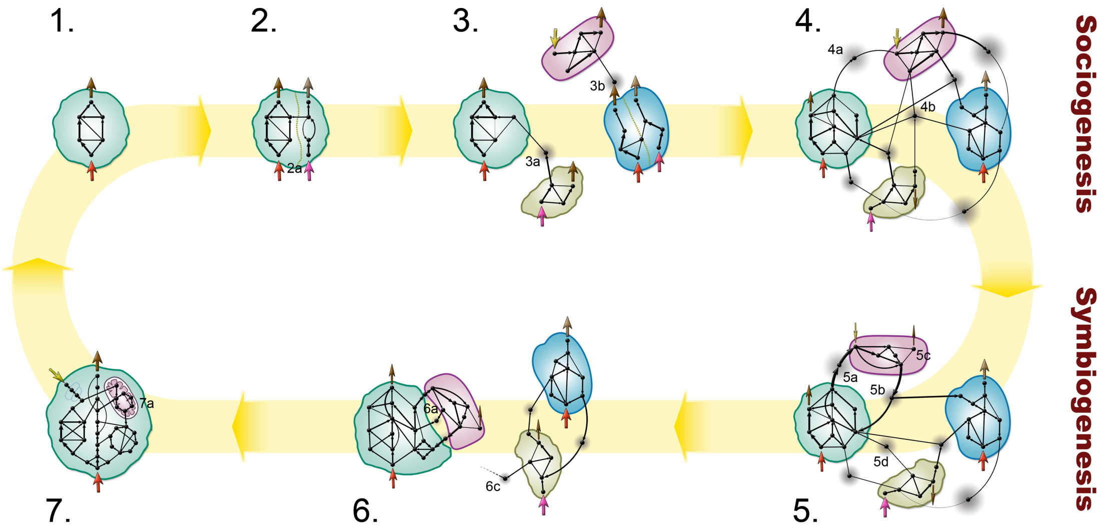

FIGURE 2 | A theoretical scenario of the evolution of a microbial facilitation network. **(1)** Microbes (colored blobs) reproduce independently with self-sustaining metabolisms. Metabolism is represented as a network of metabolites (black dots) and reactions (black arrows) indicating the main directionality and weight of the metabolic flow. Inflow and outflow are depicted as arrows pointing in and out of cells, respectively. The yellow background cycle represents a possible evolutionary route; note that the route can branch or reverse at any point. **(2)** Phenotypic plasticity allows the organisms to adapt to environmental changes. E.g., mutually exclusive metabolic pathways within an individual (step 2, separated by a dotted line) can utilize different resources efficiently, but not both (differently colored inflows at 2a). **(3)** In a community, different species may specialize on a resource while relying on community members for the utilization of other resources. According to the Black Queen hypothesis, metabolic division of labor can evolve when the shared metabolism is partitioned and distributed among species that complement each other via diffusible, leaky products (gray nodes between cells indicate diffusion of released products; 3a, 3b). **(4)** Selection can further increase cross-dependency, resulting in a multi-context division of labor with several types of mutual dependencies (i.e., nutritional, protection). Public goods can be exclusively used by two species (4a), or by anyone in the community (4b). **(5)** Symbiosis may emerge if two organisms separate from the community by relying less on public goods (5d), by downregulating pathways relying on third party resources (5c), and strengthening reciprocal cooperation by increased production rates of compounds that benefit the partner (5a, 5b). Such compounds can still serve as public goods (5b) giving rise to free-riders. **(6)** Partners can secure a more efficient exchange to exclude free-riders by privatizing resources (6a). Symbiotic pairs can gradually decouple from the community (6c) which may still provide a stable environment for them. **(7)** The transition in individuality becomes complete when no partner can reproduce alone anymore (e.g., endosymbiont gets fully integrated; (7a). The new unit of evolution acts as a single individual, and the cycle may continue at step 1 (ignoring its higher internal complexity). Ecological and evolutionary timescales are not separated.

true, bounded reproducing units (MLS2) (Damuth and Heisler, 1988; Szathmáry, 2015; Szilágyi et al., 2017). The point is that groups of entities (biofilms) may be under selection while they may not necessarily correspond to evolutionary individuals in the conventional sense (cells). Population or spatial structure may ensure that such entities reappear better than random from time to time *and* can acquire and inherit adaptations (i.e., can maintain information). If so, they may evolve to be *bona fide* groups of selection, i.e., real units of evolution in the Maynard Smithian sense (Maynard Smith, 1987), realizing an evolutionary transition in individuality (West et al., 2015).

While biofilms are likely not individually replicating units of evolution, their members may be subject to selection at multiple levels. Community-level properties can be selected for and inherited in lab environments (Hansen et al., 2007; Ren et al., 2015), which may happen in nature, given strong selection at a higher level. Multilevel selection facilitates cooperation that further benefits the collective. Ultimately, there may just be some evolutionary potential in a biofilm. The question is then, what is the information that is stably reproduced from time to time by the community? Clarke claims, that “there will rarely be enough genetic heritability across biofilm generations to support a response to selection” (Clarke, 2016). This claim needs to find support from explicit statistical measurements of the heritability of potentially adaptive community-level traits. Investigating inheritance (if any) of biofilms or lichen thalli might provide insight on how multispecies communities evolve mechanisms to ensure group selection and inheritance. Something that can and should be done in the lab.

Community Interaction Pattern as a Replicator

A phylogenetically independent metabolic profile is present in all sorts of natural and synthetic microbial communities (Burke et al., 2011; Louca et al., 2016, 2018; Goldford et al., 2018; Cui et al., 2020; Estrela et al., 2022) and potentially in non-microbial communities like higher-level ecosystems (Veldhuis et al., 2018) and biogeochemical cycles (Levin, 1998; Braakman et al., 2017). In other words, the functional profile of microbial communities seems to be generally more stable or ecologically resilient than are their taxonomic compositions (Doolittle and Inkpen, 2018). Most of such communities develop in a successive manner, where species with fitting functional capacities appear as a niche emerges, but the specific metabolic functionality rather than a specific set of species, is required in the process. This is akin the idea of guilds: a set of different taxonomic units that occupy the same metabolic niche (i.e., has the same biochemical capacity

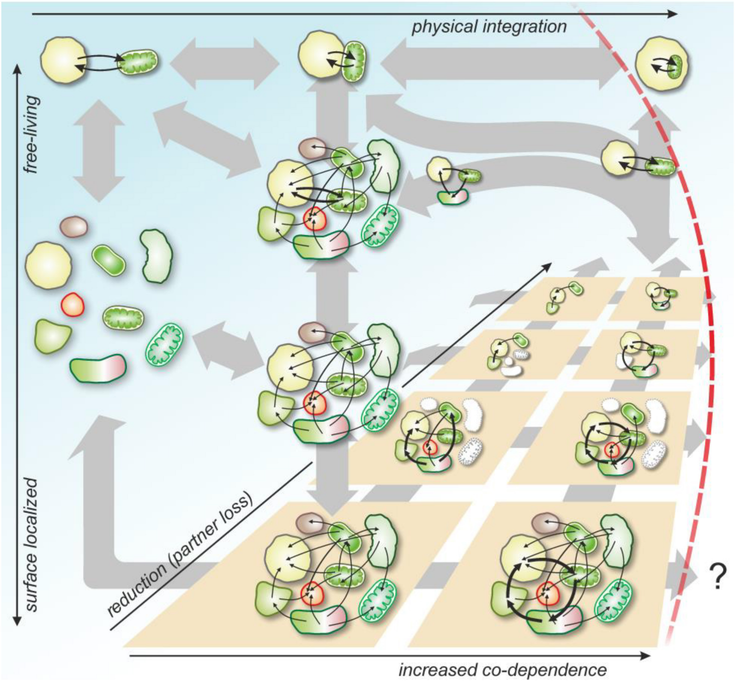

FIGURE 3 | Possible routes for microbial communities to achieve transition in individuality. Communities may be either surface bounded (brown panels) or not (blue phase). Metabolic, cooperative, and inhibitive interactions evolve between members (for sake of simplicity, arrows denote unspecified positive ecological interactions). Certain interactions may strengthen, if selection favors them (thicker arrows), certain partners may be lost due to replacement, competition or instability (grayed out cells). A symbiotic pair may emerge from free-living cells, from a loosely coupled metabolic network or from a surface-bound biofilm. The organized spatiotemporal structure of biofilms bound to surfaces may facilitate co-dependence and the emergence of cooperative cycles (or pairs), that can be selected for at the biofilm level (if biofilms compete as lineages). A transition in individuality (red separator) happens when integration of the endosymbiont becomes irreversible and the pair can only reproduce together. Alternatively (and putatively), a biofilm (or any community) may evolve means to stably replicate as a group and waive the replication of its independent cells (question mark). Ecological and evolutionary timescales are not separated.

(Burke et al., 2011)) *and* has the same competitive ability (Estrela et al., 2022). Guilds contain substantial taxonomic variation, independent of their initial starting composition (Goldford et al., 2018; Leventhal et al., 2018), due to multiple causes, such as the founder effect (random sampling from the initial species pool into new habitats) (Goldford et al., 2018), neutral community dynamics due to individual guild-member species being equivalent functionally and fitness-wise (Aguirre de Cárcer, 2019), dynamic multistability in population dynamics, and the existence of alternative stable states (Fukami, 2015) potentially caused by mutual exclusion (Leventhal et al., 2018). There is an ongoing debate about the role of selective effects vs. neutral factors during microbial community assembly (Rosindell et al., 2011; Cira et al., 2018). While community structure may be shaped by deterministic factors, such as competition or speciation, structural patterns can also be explained by neutral forces, such as stochastic birth and death processes and chance driven immigration (Hubbell, 2006; Sloan et al., 2006; Rosindell et al., 2011) or by a combination of the two types (Stegen et al., 2012; Cira et al., 2018). While high variability is often observed at the species level, there appears to be a consistency in the topological structure and in the community composition focusing on higher taxonomic levels (Goldford et al., 2018).

The phylogenetically conserved functionality of guilds (Estrela et al., 2022) provides an advantage when colonizing new habitats by providing robustness against stochastic partner loss and allowing communities to self-assemble given *any* member of the required guild is available. However, it also means that community members are not *specifically* required and guild-members could replace each other. While the global interaction network is built up more deterministically, the particular species that take the functional role may be driven neutrally. Moreover, in general, negative interactions (competition, antibiosis) tend to reduce the potential diversity of colonizers, while positive interactions (producing public goods or ecosystem engineering) are thought to increase it (Canon et al., 2020; Ratzke et al., 2020). It is often the colonization sequence of certain functional types that shapes the topology and not the set of species (Stephens et al., 2015). In general, microbiomes are characterized functionally rather than taxonomically, and while the overall structure, metabolic network, guild ratios are similar, the specific composition is not reproduced stably (**Figure 4**).

But then why would biofilms be considered replicators on their own right at all? The only requirement for a biofilm to be a higher-level replicator is that it stably reproduce *some relevant aspect* of the community in successive generations, presumably due to some form of autocatalytic multiplication. The interaction

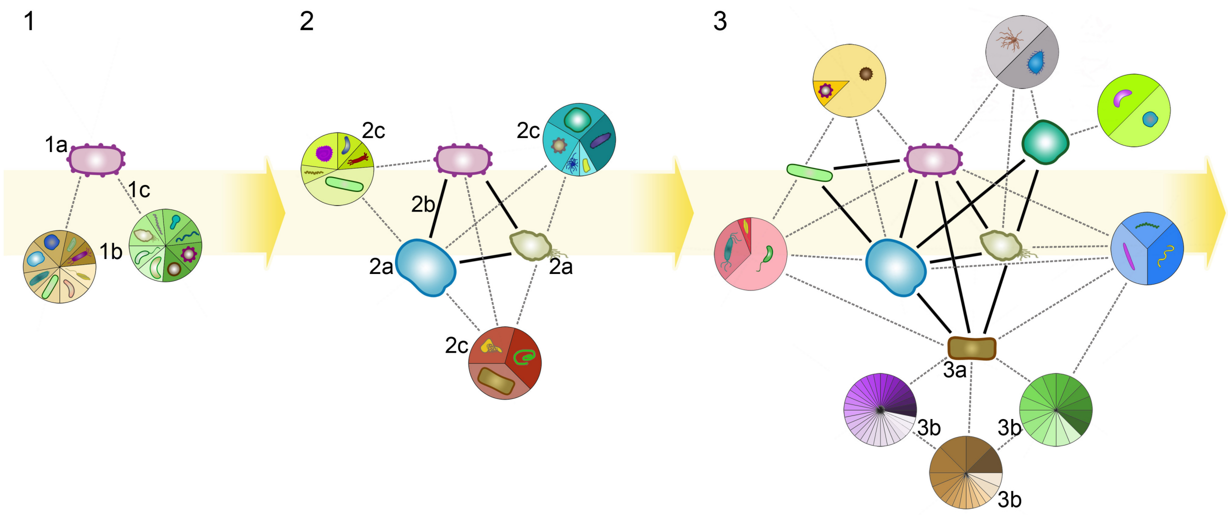

FIGURE 4 | A potential scenario of microbial community assembly driven by deterministic and neutral factors. The colonization of a new habitat may set off from a specific species (purple founder cell). Options for subsequent partners may have a wide range (pie charts), that correspond to functional types or guilds (color schemes) that fit into the emerging interaction profile (dotted lines). The particular species recruited from a guild can be due to random chance. Initially, many alternative pioneers are possible (1b), giving rise to multiple alternative topologies (not shown). Once a recruitment happens (2a), the interaction is realized (2b; solid lines). After integration, some species can be exchanged (by another species from the same pie chart) without changing the community topology or functional profile. As species gets integrated into the community (1a, 2a, 3a), the number of further options often declines (2c), as the community presents an increasingly specific environment to join into. On the other hand, a new species (3a; e.g., ecosystem engineers) may create a local environment favorable for many new species (3b).

pattern is possibly such an aspect as it certainly persists for a long time, causally derived from the biofilm, and it may inherit changes. In this regard, this approach is very similar to the replicator definition of Godfrey-Smith, where the copy of a replicator is defined as something that is causally derived and is similar to the parent "*in some relevant aspect*" (Godfrey-Smith, 2000). This aspect does not have to be subjectively specified. It is the selection process that differentiates between classes of objects, where class members are equivalent to each other *under selection*, i.e., has the same fitness value in the given local time and space (Zachar and Szathmáry, 2010). The *difference between classes* defines the variability that should be inherited. If a reproductive system can inherit variants through successive generations of autocatalytic growth and selection, then the set of variants (classes) is, by definition, the "genotypic information" of this replicator system (Zachar and Szathmáry, 2010). For example, the functional profile of a community may be the property that is selected for; all else that is unseen by selection (e.g., the taxonomic composition of the community) is not expected to be heritable and exhibit neutral dynamics (subject to phenotypic exchange or plasticity). Taxonomic composition thus cannot be part of the genotype of the higher-level replicator. The question is, then, whether there is indeed a higher-level heritable "genotype" that can be *stably maintained for an indefinite time* and whether it *can be selected for*?

In the recent decade, Ford Doolittle has elaborated on the idea that something related to the coupled metabolism of species replicates in a metabolic community, other than the exact taxonomic composition (Doolittle, 2013). Clarke (2016) has pointed out, that neither the species composition nor the metabolic capacity of the community is constant in time, changing with the environment or due to internal mechanisms (Doolittle and Booth, 2017). Biofilms may inherit not only the metabolic profile but a characteristic community interaction pattern or "interactome" (the joint metabolism, developmental interactions and phenotypic functionality of constituents). It is responsible for the development of the same functional composition (set of guilds) at subsequent habitats (Doolittle, 2013; Doolittle and Booth, 2017). Do such patterns show heritable variation in fitness that justifies considering them replicators?

According to Doolittle (Doolittle, 2013; Doolittle and Booth, 2017), the crucial mechanism that ensures replication at the biofilm level is not vertical inheritance of biofilm lineages, but the adaptive, genetically encoded recruitment of species (or guild-members) and lateral gene transfer. The latter may ensure the dispersal of adaptive genes within the guild or community (Song et al., 2021). Selective recruitment may emerge from co-aggregation mechanisms (partner recognition and preferential association and adherence (Katharios-Lanwermeyer et al., 2014)) and niche transformation. As the niche is successively transformed by the successive cooperative communities (Gatti et al., 2018), such mechanisms may more effectively regulate what species (or functional guilds) and in what order can join the aggregation. Ultimately, these traits, encoded genetically in member species, may increase the probability of co-occurrence (Periasamy and Kolenbrander, 2009; Clarke, 2016). Goldford et al. (2018) have found in the lab that starting bacterial communities self-assembled into stable multispecies communities, stabilized by cross-feeding, regardless of a high taxonomic variance in composition.

Lateral gene transfer occurs frequently in biofilms (Song et al., 2021). Constant gain and loss of genes result in a distribution of the essential *pan-genome* between strains of the community (Fullmer et al., 2015), in which all members carry a set of core genes, and only a fraction carry accessory genes (Fullmer et al., 2015; Booth et al., 2016). The pan-genome is therefore the shared genomic resource and the associated cooperative meta-cell interaction network (Fullmer et al., 2015). Several mechanisms are hypothesized to shape pan-genomes, such as gene-gene or gene-by-environment interactions, positive and negative frequency-dependent selection, or the Black Queen mechanism (Domingo-Sananes and McInerney, 2021). Indeed, a considerable proportion of microbes have disproportionately small genomes with limited metabolic capacity (Booth et al., 2016; Stolz, 2016) hence must rely on obligate partners. Such evidence puts light on the distributed nature of heritable information in microbial communities and may question how we define 'cellular' life and mutual dependency (Stolz, 2016).

As a speculative conclusion, such communities may exhibit a form of *reproduction*, from colonization to colonization, as they reproduce not only the metabolic pattern of the community, but also the pan-genome (for some extent) and succession order and the (terminal) ratio of functional guilds (Estrela et al., 2022). Species do this by relying on genes in members of the broader phylogenomic group of a guild, encoding responsible metabolic and niche-constructing traits (Odling-Smee et al., 2003; Laland et al., 2016) other guilds may benefit from. This way the metabolic effects exerted on the environment (leaked metabolites, transformed external resources) become cooperative interactions as species realize cross-feeding not necessarily simultaneously but successively in time, as the community assembles and develops.

The succession of guilds and species may yield multi-stable population dynamics with alternative stable states within a functional guild (Estrela et al., 2022). This results in different attractors of community composition, and the evolutionary route depends heavily on initial conditions and perturbations. If initial and transient stages can be stably inherited (which we do not know), then the community-level information (e.g., functional profile) is not maintained by taxonomic composition or by a conservative storage (as DNA stores genetic information), but in the attractors of the dynamical system including populational, aggregational, and environmental conditions. The added novelty, in case of microbial biofilms, is that communities themselves modify their environment as they successively develop, on a much faster timescale. Experiments show that a niche constructing strain might have higher fitness in a self-modified environment than in the original environment (Callahan et al., 2014). Genetic traits encoding for ordered niche construction are directly responsible for the canalizing effect during the succession of guilds and maturing of biofilms. They may even spread during colonization (or via LGT) and are selected for, as they ensure that the same guild succession-pattern emerges.

Ecosystems (microbial or above) can persist, reappear and function stably for a long time, maintaining a homeostatic local environment. In one dominant view, biotic feedback cycles (autocatalytic networks (Gatti et al., 2018)) can drive persistence-based selection (Lenton et al., 2021). Such feedback cycles include unrelated components performing different functions. Such cross-cooperation may allow the expansion or emergence of niches (Gatti et al., 2018, 2020) as was demonstrated in microbial communities (Großkopf et al., 2016; Gatti et al., 2018; San Roman and Wagner, 2018; Oña et al., 2021). For physiological adaptations to happen in co-evolution, prolonged stability of the environment is required, so that ecological and evolutionary contexts (balance of benefit and cost) are kept constant (Herre et al., 1999; Gomulkiewicz et al., 2003; Hillesland, 2017; O'Brien et al., 2021). A homeostatic microbial ecosystem may provide a temporal buffer for co-evolutionary processes (Bateman, 2020).

Symbiotic Pair as a Replicator

There is another option for microbes to transit from a multispecies shared metabolic community to an integrated new unit of evolution. A real example of egalitarian multicellularity, albeit not in the conventional meaning of multicellularity, is obligate endosymbioses (Szathmáry, 2015). Internalization of a symbiont involves many problems the partners are likely not preadapted for [selfishness, asynchronous cell cycles, transport, see Zachar and Boza (2020)]. Microbial partners can nevertheless benefit form synergies (see the many prokaryotic (Zachar and Boza, 2020) and protist ectosymbioses (Husnik et al., 2021)). Such partnerships may ultimately physically integrate to escape competition at the lower level (**Figure 3**). It must be noted, that waiving independent replication often occurs for the (bacterial) symbiont only (Sachs et al., 2011), which may indicate that integration has not been concluded. In *obligate endosymbiosis*, (*i*) no species can leave or reproduce without the other; and (*ii*) both species retain their metabolic identities without being eaten or degraded. There are endosymbionts which engage in syntrophic interactions with their hosts (e.g., H_2-scavanging methanogenic archaea of anaerobic protists (Wagener and Pfennig, 1987; Fenchel and Finlay, 1992; Gutiérrez et al., 2017; Beinart et al., 2018; Lind et al., 2018), sulfate-oxidizing ε-proteobacteria that colonize their surface (Edgcomb et al., 2011)).

Microbial endosymbiosis to protist hosts is diverse and ubiquitous (Lowe et al., 2016; Husnik et al., 2021), as one would expect, due mostly to phagocytosis (Nowack and Melkonian, 2010; Speijer, 2021). However, it is unknown if any eukaryotic nutritional endosymbiosis arose from an initially mutual partnership, either as a separate pair or part of a larger syntrophic community. In more general terms, we do not know from either *in vivo*, *in vitro* or *in silico* examples, if a multispecies metabolic community (especially prokaryotic) has ever given rise to endosymbiosis (Zachar and Boza, 2020) or at least to exclusively pairwise syntrophic symbiosis. Neither do we know for certain, if any microbial endosymbiosis has ever originated from an initially mutually syntrophic pair. While prokaryotic cross-feeding (communities) are ubiquitous, no prokaryote is capable of phagocytosis. Hence the possibility of endosymbiosis among prokaryotes and the origin of mitochondria remains a conundrum.

The single presumed example of endosymbiosis among prokaryotes is the possible syntrophic origin of mitochondria, according to syntrophic theories (Martin et al., 2015; Spang et al., 2019; Imachi et al., 2020; López-García and Moreira, 2020). These theories posit an initially mutual metabolic cooperation between the ancestors of mitochondria and host, instead of exploitation by parasitism (White et al., 2018) or by phagocytosis (Martijn and Ettema, 2013; Zachar et al., 2018). Syntrophy ultimately lead to the unspecified engulfment of the bacterial partner. Such an inclusion between syntrophic prokaryotes (or protists for that matter) has never been documented. On the other hand, the sheer amount of syntrophies among prokaryotes proves that mutual syntrophy is an extremely common and stable interaction *when external*. Protists either receive their numerous endosymbionts via eukaryote-specific ways (via phagocytosis) or via parasitism, which are both well documented (Sachs et al., 2011; Speijer, 2021). The primary plastid of all plants probably had a single origin (Adl et al., 2018), at which point the host was likely already phagocytic, even if only rudimentarily (Mills, 2020). Later re-uptakes of plastid-bearing cells by protists sometimes retained the phagosomal membrane as a proof of phagocytic inclusion (Keeling, 2013; Speijer, 2021). On the other hand, according to a common view, the vast majority of extant host-bacteria associations are mostly parasitic of origin, and are costly for the host (Sachs et al., 2011; Keeling and McCutcheon, 2017; Husnik et al., 2021). If syntrophy has indeed enabled and facilitated endosymbiosis of mitochondria, one must ask, why such transitions are not seen more often among prokaryotes?

We hypothesize that the lack of (endo)symbiotic examples originating from a syntrophic community is due to multiple factors. For eukaryotes, it is mostly because those protists capable of phagocytosis (i.e., has a mechanism to integrate a symbiont) are never participating in biofilm formation or syntrophic communities. Parasitic endosymbionts capable of entering a host by themselves prefer heterotrophic hosts for energetic reasons, again not members of biofilms. The issue is clearer in case of prokaryotic symbioses, where phagocytosis is not an option to acquire the partner. We do not know how many of the pairwise syntrophic ectosymbioses of prokaryotes emerged from larger communities, but there is no example of *bona fide* endosymbiosis other than possibly mitochondria (Zachar and Boza, 2020), and even in that case it is not clear whether they started out as an exclusive pair or part of a community. The cause must be inherent to metabolic network topology and stability of communities (prokaryotic and eukaryotic alike in this regard).

The alternative hypothesis, at least for eukaryotes, is that endosymbiosis does happen more frequently in larger communities, but then the pair quickly decouples from or dominates the network due to the pair benefiting the most under selection (**Figures 2**, **3**). A third option is that syntrophic endosymbioses do not come from syntrophic communities. They may have evolved from syntrophic pairs that were never part of a bigger community; or syntrophy may evolve *after* integration (like the ATP-ADP exchange of mitochondria and host, as is believed so (Zachar and Szathmáry, 2017)), assuming the initial interaction was not mutualism (Zachar et al., 2018). These hypotheses could be tested, by modeling the emergence of pairwise symbiosis within or without communities and testing their stability against internal and external perturbation.

One may ask, why exclusively pairwise interactions are not more common in microbial communities. Syntrophic mutualism is the microbial realization of a 2-membered cooperative cycle where both species benefit from the interaction. A natural extension then is to assume that a 3 or N-membered cooperative cycle can also emerge, stabilize and be selected for at the group level. However, no multimembered circularly cooperating microbial metabolic network is known. There exist communities with cyclic cooperation (e.g., 3-species *Utricularia* system (Ulanowicz, 1995; Ulanowicz et al., 2014)), but microbes usually form more complex networks: positive interactions are not necessarily reciprocated (directly, specifically or exclusively) and a cooperative chain is not guaranteed to close. Most importantly, direct interactions are often competitive (even inhibitive) leading to complicated interaction networks, that can still lead to community stability (Kato et al., 2005). Circular competition is common, but exclusively circular mutualism is rare, possibly nonexistent, for $N > 2$. Naturally, the larger a cooperative cycle is, the more prone it is to selfish cheaters and destabilization. It is unknown (and unlikely) if such a network can give rise to a specific mutual pair that can stably emerge, without strong means of vertical co-inheritance (like engulfment).

In summary, it is yet unknown if endosymbiosis could or have ever evolved in multispecies communities from mutual syntrophy. Based on observable data, we believe that, on one hand, **endosymbiotic integration of mutually syntrophic prokaryotes is extremely rare**. On the other hand, **protists likely acquire their endosymbionts via pairwise interactions (e.g., phagocytosis) and not as being part of a community or biofilm**. This, of course, does not mean that any of it is impossible. The singular case of long term stable endosymbiosis emerging from mutual syntrophy, as theorized for mitochondria, could be the result of an extremely improbable event.

CONCLUSION

We have reviewed how microbial cooperation can emerge and stabilize communities and how they can potentially facilitate a major transition. We pointed out, that the **metabolic topology** (cross-feeding) itself is not enough to analyse cooperation, as it only defines a trophic interaction mediated by metabolites. One must know the **cooperative topology**, the second order catalytic aid that provides a benefit. We reviewed how cooperation may emerge via metabolic interactions and public goods in microbial communities, and have considered the theoretically possible routes how a microbial community may achieve higher integration due to cooperation and transition in individuality. Finally, we have investigated how a multispecies community may undergo group selection and inherit community-level variations.

If biofilms could somehow undergo a transition in individuality, there would be a theoretically possible escape route from the microbial hurdle toward higher level integration and a major transition, potentially available for prokaryotes too. Admittedly, some of these ideas are speculative, but they serve a dual purpose. Firstly, they clearly distinguish between the various aspects of a community that may or may not carry heritable information. There may be some epigenetic information (even if limited) encoded not in genes, that can be subjects of selection at the level of the collective. Secondly, they identify the possible routes that may be more realistic in other domains, where similarly coupled Darwinian systems exist. Biofilms may be a domain-specific case of a more general idea of replicators coupled through (metabolic) facilitation, and while they may have at best limited heredity that we know of, they may have a potential for increasing this limit or facilitating a possible future transition (Robin et al., 2021).

Certain features of a biofilm do contribute to the growth, survival, and multiplication of the community. While total information content of a community likely cannot reappear deterministically from time to time, some subset of this information may do so, despite severe ecological and evolutionary fluctuations. One can even state, that these features, responsible for this subset of information, are replicators as they re-appear with the biofilm. Indeed, the extended phenotype (the interaction pattern or the constructed niche of the biofilm) may be autocatalytically generated and may cross-catalytically help the biofilm. There is certainly a positive feedback loop (catalysis) between the properties of the biofilm and biofilm species (their genes) that benefit the biofilm as their extended phenotype, leading to positive selection. However, autocatalysis should not be mistaken for informational replication: biofilm-level adaptations do not inherit their variations. Without variability that can be causally and stably reproduced, these extended cycles cannot inherit information and are thus non-informational replicators (Zachar and Szathmáry, 2010). The nest of the bird, the dam of the beaver, the habitat of microbiome may catalyze the replication of their respective replicators (bird, beaver, microbes), but they cannot stably inherit variations.

There may be properties of a biofilm, that are replicated from time to time as the community spreads (guild amounts, ratios, metabolic capacity, interaction pattern, constructed niche, etc.). Can any of these inherit changes? This can only be ascertained if one measures the response to selection and the heritability of community traits of a biofilm "population" on a statistical basis. We must emphasize, that niche construction, causing persistent conditions of the external environment, should only be considered if persistent external entities can pass on acquired variation. In reality, variations of the external conditions likely cause the extinction of the community or won't affect its replication: while there may be a causal arrow, there is no informational arrow from habitat to community.

Recently, there have been advances in artificial engineering and directed selection of microbial communities (Castle et al., 2021). Artificial community selection aims at finding a proper map between community composition and community function, to improve desired functionalities via selection (Chang et al., 2021). Theoretical models present promising results regarding the heritability of community functions (Xie and Shou, 2021), however, success in establishing long-lasting modifications in engineered functionalities are yet to be seen in experimental systems (Albright et al., 2021). Note, that when the environment repeatedly enforces a single outcome (as often does under artificial selection), it should not be called replication, as relevant variations are not inherited.

Is there a chance for a major transition in a *multispecies biofilm* then? Certainly. One can imagine directed selection in the lab that enhances existing mechanisms of association and prefers novel adaptations that further ensures better together-replication of partners against competing biofilms. Could it happen in the wild? We see no theoretical objection to it. Is there any such living microbial community that have been transited or are in transition? Not that we know of. But there are microbial communities that reappear more frequently than others from time to time (especially, when the species count is as low as 2; explored in Zachar and Boza, 2020), indicative of their reproductive and associative stability. Has it ever happened to a biofilm? Not that we know of. At least not in the sense one would imagine the compartmented replication of groups of microbes of the community. One can argue, that if syntrophy can lead to the integration of individually replicating species, as mitochondriogenesis theories assume, then it may provide a particular route how aggregative evolutionary transitions may happen in the microbial world. On the other hand, the observed fact that metabolically coupled communities fail to transit to higher levels of selection and form *bona fide* groups may be an indication that this transition is exceptionally hard, at least in the prokaryotic and protist domains. Maybe, there are other domains where formally analogous systems can form and have better chances to achieve a transition.

Doolittle and Booth (2017) claim, that interaction patterns do vary and that their variation is heritable and causes differential fitness, leading to the evolution of these networks by natural selection. That is, there is an "epigenetic" source of heritability in biofilms, that could support evolution of the biofilm at a group level. It may turn out that syntrophic communities in this regard can be considered informational replicators, encoding *something* about their dependent metabolism and interactions at the group level. Likely, this information has limited variability (the amount of selectively different attractors defined by different functional profiles), hence biofilms may only have limited hereditary potential (Szathmáry, 2000). If this is so, we hypothesize, that the transition from this limited hereditary state to unlimited heredity is not possible due to the attractor-based inheritance system of the community. There is just not enough variability for evolution to select for and improve upon. There is, however, a lack of modeling to support this at the moment.

But let's assume for a moment, that some information is heritable. The biofilm thus likely have at least limited heredity. If there is heredity, there is differential selection of the variants. If there is selection, there may be adaptations that affect the collective and are selected for. Epigenetic systems do exist, complementing genetic inheritance (Jablonka and Szathmáry, 1995; Jablonka et al., 1998). They also have limited heredity, so there is nothing unorthodox about assuming that a (microbial) community may pass on aggregate-level information to new colonies. At this point we do not claim that any community property qualifies as a heritable property, we only claim that we do not see a theoretical objection that such things may or may have existed. Whether such limited heredity would ever become practically unlimited and whether it would enable a classical transition in individuality are unlikely due to the competitive, distributed and highly variable nature of microbial communities. If, however, one accepts that such properties could exist, one has to follow the rabbit hole all to the end, to the full Darwinian suite that leads to open ended evolution of a new level of informational inheritance system and thus to a potential major transition.

AUTHOR CONTRIBUTIONS

IZ and GB contributed to the writing and the editing of the manuscript and the designing of the figures. Both authors contributed to the article and approved the submitted version.

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REVIEW
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Developmental Integration of Endosymbionts in Insects

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In endosymbiosis, two independently existing entities are inextricably intertwined such that they behave as a single unit. For multicellular hosts, the endosymbiont must be integrated within the host developmental genetic network to maintain the relationship. Developmental integration requires innovations in cell type, gene function, gene regulation, and metabolism. These innovations are contingent upon the existing ecological interactions and may evolve mutual interdependence. Recent studies have taken significant steps toward characterizing the proximate mechanisms underlying interdependence. However, the study of developmental integration is only in its early stages of investigation. Here, we review the literature on mutualistic endosymbiosis to explore how unicellular endosymbionts developmentally integrate into their multicellular hosts with emphasis on insects as a model. Exploration of this process will help gain a more complete understanding of endosymbiosis. This will pave the way for a better understanding of the endosymbiotic theory of evolution in the future.

Keywords: bacteriocytes, developmental integration, Hox genes, interdependence, ecology, evolutionary novelty, endosymbiosis

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INTRODUCTION

Endosymbiosis is an association between different species where one lives inside the body of another, often involving a mutual benefit (Buchner, 1965). There are a multitude of forms of endosymbiotic relationships, in the case of both unicellular and multicellular organisms. The endosymbionts can be intracellular or extracellular, they can be obligate or facultative, they can be mutualistic, commensalistic, or parasitic (Gupta and Nair, 2020). Here we focus on the mutualistic endosymbioses, although some of the concepts are applicable to commensalism and parasitism, a discussion on those is beyond the scope of this review.

The processes involved in maintaining an endosymbiotic association must conform with the native molecular genetic processes of the host. In multicellular organisms, the genotype and environmental inputs are brought together by the process of development to give rise to a phenotype, which is subject to evolution (Abouheif et al., 2014; Gilbert et al., 2015). Development, in its broadest definition, is the process of progressive and continuous change that generates a complex multicellular organism from embryogenesis, maturation to senescence (Gilbert and Barresi, 2017). Therefore, understanding how a unicellular endosymbiont integrates with the biology of its multicellular host requires the study of development of the multicellular host in the context of endosymbiosis (Gilbert et al., 2015).

Whether the endosymbionts are maternally transmitted or acquired later in life, development guides interactions between the partners in ways that intertwine the endosymbiotic association with the developmental process (**Figure 1**). The infection of endosymbionts into the host is highly developmental stage-specific while the phase of the life cycle during which endosymbionts remain associated with the host is tightly regulated (Koga et al., 2012; Catta-Preta et al., 2015; Russel et al., 2017). The stage at which endosymbionts multiply within the host tissue is also under strict control and the population size and diversity of the endosymbionts are regulated by the developmental process (Wolschin et al., 2004). The movement and packaging of endosymbionts within the host body are characteristic of any given host-endosymbiont pair and specific cell types to harbor endosymbionts are uniquely present in particular hosts (Braendle et al., 2003; Stoll et al., 2010; Ratzka et al., 2012). Endosymbionts may influence and be affected by the process of metamorphosis, which also causes the removal of the gut microbiota between specific stages, while the cellular endosymbionts are retained (Hammer and Moran, 2019). Remarkably, loss of endosymbionts leads in some cases to developmental alterations that are lethal or costly in terms of reproductive success (Schwemmler, 1974; Rafiqi et al., 2020). The exact manner in which development coordinates between endosymbiont-host interactions are in the early stages of exploration at the experimental level and the theoretical framework for the involvement of development is lacking.

In the sections that follow we first summarize the current understanding of the nature of inter-relations between organisms that engage with each other. We then discuss the genomic changes underlying these inter-relations. Taking into account the biological development of multicellular hosts, we explore the phenomenon of developmental integration of endosymbionts with their hosts. We suggest developmental integration as the driving process that coordinates genotype and inter-organismal relations to potentiate, originate, and maintain endosymbiosis, by means of which free-living bacteria become endosymbionts of multicellular hosts.

INTERDEPENDENCE BETWEEN ENDOSYMBIONT AND HOST

When free-living bacteria transition to being mutualistic endosymbionts, they start to influence the host in multiple aspects, which make either the endosymbiont or the host-dependent on the other in nutrition, metabolism, immunity, genetics, ecology, or a combination of these (Baumann, 2005; Moran et al., 2008; Flórez et al., 2015; Masson et al., 2016; Rafiqi et al., 2020). This dependence can be unidirectional or bidirectional, and potentially evolve from one to the other. Moreover, the changes leading to interdependence may occur prior to-, along with-, or after- the initial establishment of the endosymbiotic association. If an interdependence arises after the initial establishment of endosymbiosis, rewiring or interruption of non-essential pathways or processes in either of the organisms may occur by evolutionary mechanisms (Wernegreen and Moran, 1999; Wernegreen, 2002; Zientz et al., 2004; Baumann, 2005; McCutcheon and Moran, 2007, 2012). It can be speculated that under the scenario of interruptions or rewiring, notwithstanding the specific genes affected, conservation of resources such as substrates of molecular reactions and energy for the partners as a whole is likely to be favored by natural selection. However, it has been shown that in the absence of selective pressure, genes involved in these pathways or processes are often lost by genetic drift (Wu et al., 2006; McCutcheon and Moran, 2012; Sloan and Moran, 2012; Wernegreen, 2015). Conversely, a progressive co-adaptation leading to interdependence arises over evolutionary time with features in the partners being retained due to the advantages for both the endosymbionts and hosts (Wu et al., 2006; Douglas, 2014; Wernegreen, 2015). Given the changes discussed above, the degree of interdependence between the host and the endosymbiont appears to be constantly evolving throughout their association.

Nutritional, metabolic, immune-related, genetic, or ecological changes are accompanied by changes at the genomic level. Interdependence between host and endosymbiont ultimately comes from genetic input, which is further aided and potentiated in two ways (Wilson and Duncan, 2015). (i) Horizontal gene transfer between genomes of endosymbiont and host leads to the insertion of new genes in the host genome (Nakabachi, 2015). Surprisingly, horizontal gene transfer is more common from facultative endosymbionts than from obligate endosymbionts (Nikoh and Nakabachi, 2009; Husnik et al., 2013; Husnik and McCutcheon, 2018). The acquisition of new genes, by horizontal gene transfer, provides an important source of innovations facilitating further evolution of the relationship between endosymbiont and host (Keeling and Palmer, 2008; Sloan et al., 2014; Blondel et al., 2020). One way in which this can occur is by substitution of obligate endosymbiont genes by horizontally transferred genes from facultative endosymbionts provided the two endosymbionts have shared homology in genes and pathways. The gain of endosymbiont genes by hosts, from a facultative endosymbiont, would make the homologs of these genes redundant in their obligate endosymbionts and facilitate the loss of these genes in the obligate endosymbiont (Wilson and Duncan, 2015). (ii) The genomes of endosymbiotic bacteria are generally much smaller when compared to their free-living relatives implying that after the establishment of endosymbiosis, the endosymbiont genome undergoes size reduction (Douglas, 1998; Moran, 2002; Latorre et al., 2005; Toft and Andersson, 2010; McCutcheon and Moran, 2012; Russell et al., 2013). Genomic changes previously discussed as accompanying endosymbiosis in many cases further embed the process of interdependence between the endosymbiont and host. Yet, these genomic changes do not necessarily co-occur with the initial establishment of endosymbiotic association but may secondarily enhance the association.

The endosymbiont and host together behave as a single ecological unit, wherein being together increases the number of ways in which they can adapt to the environment or allows them to occupy specialized niches such as nutrient-poor diets (Buchner, 1965). The endosymbiont genome provides an additional source of heritable information that has the potential to impact the endosymbiotic association. As a whole, host-endosymbiont interdependence can be perceived as an intricate interplay of biological entities that impact all the levels of

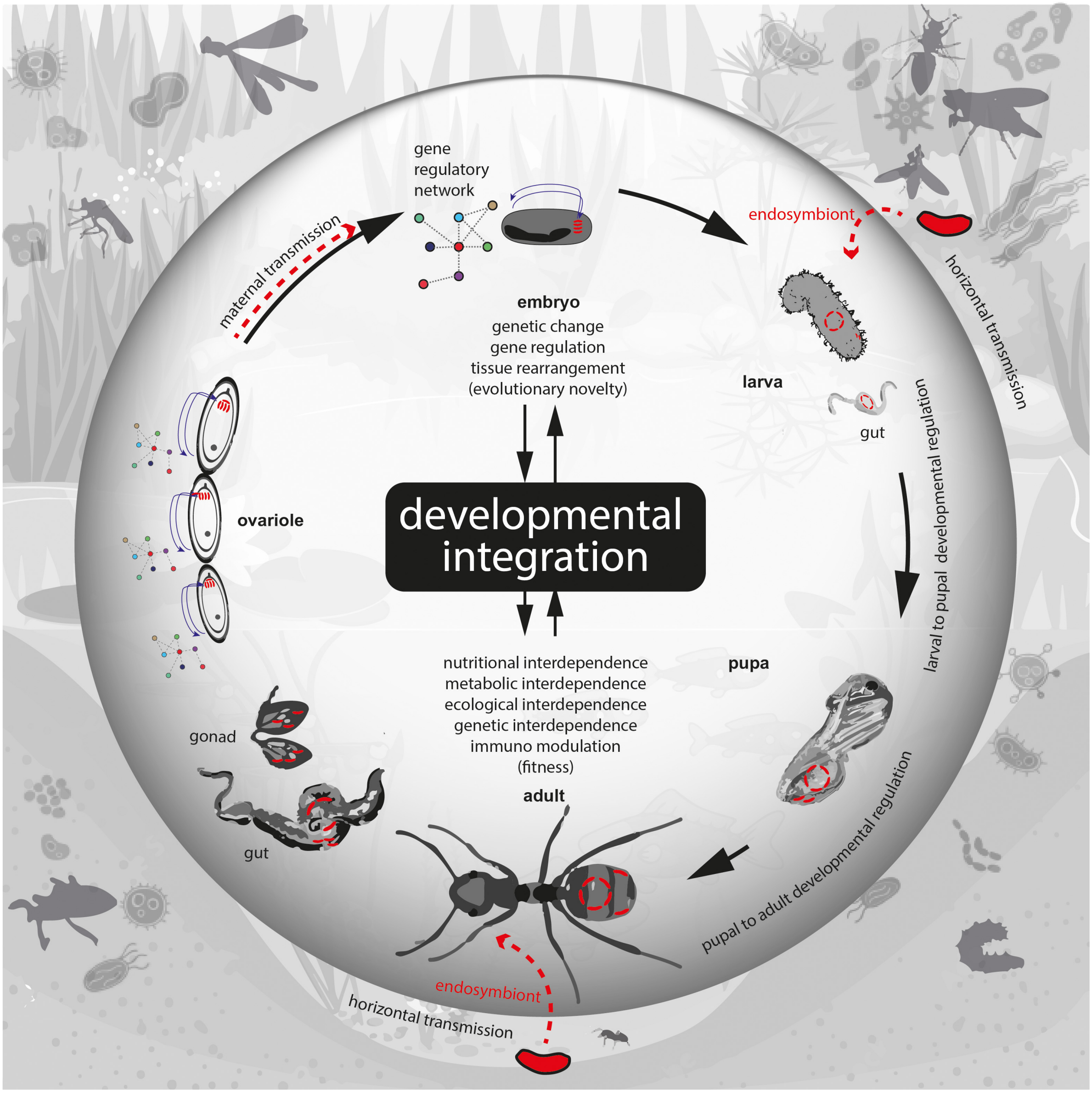

FIGURE 1 | Illustration showing developmental integration in the process of maintenance of endosymbiotic association between a bacterial endosymbiont and an ant host as an example. Bacteria are shown in red and bacterial cell walls in black. Different developmental stages are shown representing the lifecycle of the ant that undergoes multiple steps of metamorphosis from egg to larva to pupa to adult. Red dotted arrows indicate transmission, which can be horizontal or vertical (maternal). Gene regulatory network is represented as colored circles connected with lines. Small arrows indicate influence, large arrows indicate metamorphic stage transitions.

biological organization (Janson et al., 2008; Feldhaar, 2011). Interdependence between the endosymbiont and host—due to nutritional, metabolic, immune-related, genetic, or ecological changes—potentiated by horizontal gene transfer, and genome reduction—results in the maintenance of endosymbiosis that ultimately may lead to an irreversible dependence on each other. The origin of interdependencies is phylogenetically contingent in that it arose from ancestors with particular physiology, that lived under particular ecological conditions, and are shaped by evolutionary forces acting on interacting partners (Rafiqi et al., 2020). Even after the symbiont becomes established as a permanent resident over time, countless other adjustments in different biological levels are triggered in both partners to accommodate each other more efficiently (Moya et al., 2008; Toft and Andersson, 2010; Perreau and Moran, 2021). Recent research has pointed out the fact that both partners in endosymbiosis "converse" at the molecular level from the early stages of host development (Banfill et al., 2020; Rafiqi et al., 2020).

Understanding the mechanisms that govern the initial establishment of endosymbiotic interdependence will require the following two approaches: One, a thorough sampling of endosymbiosis-related phenotypes in a vast number of related organisms. Two, a detailed comparison of host molecular genetic processes between organisms that contain endosymbionts and their closest relatives that lack them. The latter approach could immensely benefit from vigorous research in the field of developmental biology in the recent decades to further understand the establishment as well as maintenance of endosymbiotic associations. This approach would involve studying host-centric mechanisms of endosymbiont transmission and bacteriocyte specification on the one hand and endosymbiont-centric mechanisms of integration into host development as well as the dynamic role of endosymbiont during host development on the other hand.

DEVELOPMENTAL INTEGRATION

In an endosymbiotic relationship, the host acquires, maintains, and transmits their endosymbiont from generation to generation or acquires the endosymbiont in a stage-specific manner during development (**Figure 1**). It is during development that the genotype and environmental factors are integrated into the phenotype through multiple interacting mechanisms. Endosymbiosis must therefore have a dynamic interaction with the developmental process. Developing organisms are under the control of multiple essential processes including the migration, proliferation, specialization, and death of cells, for producing new cells with different characteristics at different locations in the organism. These cellular processes are achieved through differential gene expression, intra and inter-cellular transport of mRNAs and proteins, and cell-to-cell communications that lead to the organization of cells into multicellular arrangements such as tissues and organs (Gilbert and Barresi, 2017). Developmental events are controlled by gene regulatory networks that compose signaling cascades and pathways (Lewis, 1978; McGinnis et al., 1984; Lehmann and Nüsslein-Volhard, 1986; Schupbach and Wieschaus, 1986; Padgett et al., 1987; Ingham, 1988; Tautz, 1988; Akam, 1989; Mann and Hogness, 1990; Shimell et al., 1991; St Johnston and Nüsslein-Volhard, 1992; Arora et al., 1994; Mason et al., 1994; Biehs et al., 1996; Tomoyasu et al., 2005; Campos-Ortega and Hartenstein, 2013). In multicellular organisms, signaling pathways require an array of chemical substances such as hormones, growth factors, neurotransmitters, and extracellular matrix components, acting locally or traveling long distances within the organism (Gilbert and Barresi, 2017). This complex and well-orchestrated interplay of events inside the developing organisms often proceeds in the presence of endosymbionts. Additionally, endosymbionts that are vertically transmitted accompany the organisms from its single-cell stage to reproduction and death. Therefore, their continued presence must increase the likelihood of evolving interactions and influences on the developmental processes.

Several questions therefore arise from this intersection of endosymbiosis with development. How do the endosymbionts interact with the host to result in stable integration of one cell into another, given the tightly regulated developmental gene networks that define the cell identities, body axes, segments, and the germline? Conversely, how does the host regulate endosymbiotic populations without affecting their function during development? Additionally, evolutionary conflicts of interest between endosymbiotic partners complicate this process because organisms most often tend to evolve in such a way that promotes their own fitness at the expense of their partner's fitness (Garcia and Gerardo, 2014; Bennett and Moran, 2015; Lowe et al., 2016; Keeling and McCutcheon, 2017). Therefore, the connection between the host and the endosymbiont is complicated and dynamic, including highly precise adaptations and counter-adaptations.

In the last three decades, there has been a lot of interest in the mechanisms of endosymbiosis (Moran and Baumann, 2000; Russell and Moran, 2006; Hansen and Moran, 2011; Login et al., 2011; Landmann et al., 2014; Weinert et al., 2015; Bennett et al., 2016; Kupper et al., 2016; Gray, 2017; Mergaert et al., 2017; Skidmore and Hansen, 2017). However, due to technological limitations, as noted by Wilson and Duncan (2015), most of the studies in the field have treated the interacting host and endosymbiont as distinct organisms. From this perspective, the two distinct genomes may appear to be thought of as interacting partners that merely communicate *via* signals. This view is incomplete because endosymbionts appear more embedded within the molecular genetic processes of the host. The endosymbionts directly influence the development of their hosts in numerous and innovative ways. For example, the endosymbiont *Vibrio fischeri* constitutes the essential light emitting cells of its host squid *Euprymna scolopes* where during its development bacterial cells are induced to become non-motile and trigger the host epithelial cells to swell for endosymbiont acquisition (Nyholm and McFall-Ngai, 2004). The bacteria release tracheal cytotoxin that acts as a morphogen influencing the development of the crypts in the epithelium that makes the light organ of its host squid *E. scolopes* (Koropatnick et al., 2004). The bacteria also release a small non-coding RNA that not only influences morphological changes in the host epithelium but also modulates the expression of host immune response genes such that when a mutant bacterium that lacks the gene for this small non-coding RNA is provided to the squid, it fails to cause the proper formation of the light organ (Moriano-Gutierrez et al., 2020).

In the aphids, *Buchnera* varies throughout clonal aphid lineages being influenced by environmental and host genetic variables. Gene expression in the bacteriocytes differs between clonal populations of aphids while *Buchnera* gene expression adjusts accordingly to the genotype of the host (Smith and Moran, 2020). Comparing hosts with low and high *Buchnera* titer shows that aphids and *Buchnera* oppositely regulate genes underlying cell growth and amino acid biosynthesis (Chong and Moran, 2016). Bacteriocytes and endosymbionts show a high level of expression of genes underlying energy metabolism in the case of high-titer aphids (Chong and Moran, 2016). Also, several cell signaling pathways of high-titer hosts such as cytokine pathways, lysosomal processes, membrane trafficking, and mechanistic target of rapamycin (mTOR) are up-regulated (Smith and Moran, 2020). Additionally, genes related to flagellar body secretion are overexpressed in low-titer hosts and those of flagellar assembly are overexpressed in high-titer hosts (Chong and Moran, 2016). Altering the diet of the aphid host also elicits changes in the expression of small non-coding RNAs of the endosymbiont target genes related to pathways involved in essential amino acid biosynthesis (Thairu et al., 2019).

In *Drosophila, Wolbachia* can influence the expression of germline genes (Fast et al., 2011; Ote et al., 2016). *Wolbachia* is found in germline tissues as well as detected in somatic tissues (Dobson et al., 1999; Cheng et al., 2000; Clark et al., 2002; Ijichi et al., 2002). Maternally transmitted *Wolbachia* persist throughout embryogenesis and are incorporated into the pole cells, which make the gonads (Kose and Karr, 1995). It has been shown that *Wolbachia* exhibits striking subcellular localization using microtubules and Dynein at the anterior pole during oogenesis (Ferree et al., 2005). This is the mechanism by which they transmit to the next generation exploiting the host's microtubule cytoskeleton and transport system (Ferree et al., 2005). In most cases, only a smaller sub-population of endosymbionts is developmentally destined to be vertically transmitted, creating a bottleneck effect for the evolving endosymbiont population. For example in the *Camponotus floridanus*, the majority of endosymbionts are steered by the developmental process to become housed in the midgut epithelium for producing nutritional benefits to the host, while as an order of magnitude smaller populations reside in tissues closely proximal to the gonads (Rafiqi et al., 2020).

The role of endosymbionts in the development of the host is highlighted by studies where experimental elimination of endosymbionts leads to significant alteration of host development. For example, in the *Euscelis* bugs, the elimination of endosymbionts leads to truncation of the abdomen indicating that the endosymbiont influences posterior pattern formation in this organism (Schwemmler, 1974). In the filarial nematode *Brugia malayi,* loss of *Wolbachia* alters the anterior-posterior pattern formation hinting at an interaction with the segmentation cascade that defines this axis (Landmann et al., 2014). In the cereal weevil, *Sitophilus oryzae*, the endosymbiont *Sodalis pierantonius* affects transcripts involved in cell apoptosis, autophagy, and gut epithelial cell swelling and delamination (Masson et al., 2015). In the Cnidarian host *Hydra*, the genome of *Curvibacter* endosymbionts produces signaling molecules N-acyl homoserine lactones that are subsequently modified by host-encoded enzymes, resulting in dramatic shifts in endosymbiont gene expression and phenotype (Pietschke et al., 2017). In the *C. floridanus* ants, the elimination of *Blochmannia* leads to loss of germline and Hox gene expression domains in the embryo that in turn causes changes in the position of the functional germline formation and influences gonad formation leading to complete loss of gonad in a proportion of the embryos (Rafiqi et al., 2020). In some cases, there is an influence on growth, development, or reproduction but the pathways through which this is achieved remain elusive (Koga et al., 2003; Hosokawa et al., 2008; Kuriwada et al., 2010; Xue et al., 2012; Hickin et al., 2022). Endosymbionts and hosts therefore affect gene regulation of each other and exhibit diverse adaptations toward survival and transmission. But how the host gene regulatory networks adapt immediately after the initial encounter with endosymbionts has scarcely been explored. A couple of studies have so far uncovered possible key players in the process, and it appears that members of highly conserved gene families are involved in the developmental integration of endosymbionts.

Hox Genes Facilitate Developmental Integration

Hox genes usually define segment identity along the anterior-posterior axis (Lewis, 1978; McGinnis et al., 1984; Lehmann and Nüsslein-Volhard, 1986; Schupbach and Wieschaus, 1986; Ingham, 1988; Tautz, 1988; Akam, 1989; Mann and Hogness, 1990; St Johnston and Nüsslein-Volhard, 1992; Tomoyasu et al., 2005). However, Hox genes of the Bithorax complex have been shown to play a role in patterning the bacteriocytes in a hemipteran bug *Nysius plebeius*, and the ant *C. floridanus* (Matsuura et al., 2015; Rafiqi et al., 2020). In the case of *N. plebeius*, a loss of function of the Hox gene *Abdominal A* (*AbdA*) leads to mis-regulation of bacteriocyte formation and that of the Hox gene *Ultrabithorax* (*Ubx*) leads to a complete loss of bacteriocytes (Matsuura et al., 2015). In *C. floridanus,* not only are the homologs of these Hox genes involved in bacteriocyte differentiation but also at the same time the expression of these Hox genes is dependent on the endosymbiont *Blochmannia* such that their expression patterns alter after removal of endosymbiont through antibiotic treatment (Rafiqi et al., 2020). Therefore, there appears to be a regulatory loop between Hox gene regulation and signals from the endosymbionts, raising the possibility that patterning components interact with the endosymbiont in these ants and perhaps in other organisms that carry and maintain such endosymbionts. Moreover, phylogenetic analysis has shown that in the *Camponotus* ants and their closest relatives within the ant tribe Camponotini, early development has been drastically changed following the acquisition of a bacterial endosymbiont in multiple ways (Rafiqi et al., 2020). Most strikingly, these ants form two germlines during embryogenesis: one germline through the accumulation of maternal mRNAs and a secondary germline through endosymbiont driven zygotic induction mechanisms *via* the activity of Hox genes (Rafiqi et al., 2020). In these ants, endosymbionts regulate the localization of mRNAs of genes that define the germline such as *oskar, vasa, nanos, tudor, aubergine,* and *staufen via* Hox genes *Ubx* and *abdA* such that these germline genes become localized in more than one subcellular locations in contrast to all other known insects where they localize to a single location of the embryo (Rafiqi et al., 2020). These results indicate that the endosymbiont brings about significant changes that affect the development of the host.

Intriguingly, the ant endosymbiont *Blochmannia* is closely related to endosymbionts present in hemipteran mealybugs

(Wernegreen et al., 2009), and homologs of the Hox genes *abdA* and *Ubx* pattern bacteriocytes in both of these diverse lineages (Matsuura et al., 2015; Rafiqi et al., 2020). Is it possible that because the two hosts have very similar gene regulatory networks, an endosymbiont may benefit from host interactions acquired in an ancestral relationship? A kind of interaction that allows the endosymbiont to recognize a highly conserved gene regulatory pathway in novel hosts would provide an advantage for integration into the host. This suggests that Hox genes may have played a role in the horizontal transfer of *Blochmannia* from mealybugs to *Camponotus*. Other highly conserved genetic pathways in diverse organisms may influence the horizontal transfer of endosymbionts. Consequently, the prospect of endosymbionts transferring to more distantly related hosts, would increase or decrease based on the properties acquired within a primary host. We speculate that highly conserved gene networks such as that of Hox genes may therefore have contributed to the establishment of endosymbiosis among a large number of multicellular organisms.

Evolutionary Novelties Accompany Developmental Integration

Evolutionary novelties are unique phenotypes with novel functions found in a specific taxon (Pigliucci, 2008). Bacteriocytes are an example of evolutionary novelty that appear to have evolved multiple times independently in different taxa and differentiate during development to accommodate the endosymbiont (Moya et al., 2008). Bacteriocytes and their resident endosymbionts may also aggregate to form an organ-like structure called the "bacteriome" (Buchner, 1965). Organs or tissues housing intracellular endosymbionts are not only restricted to insects but also found in their closest relatives: trophosome of deep-sea tubeworms (*Annelidae*) or the gill filaments of lucinid bivalves (*Mollusca*) (Cavanaugh et al., 1981; Frenkiel and Mouëza, 1995). These organs develop either from mesodermal tissue or from undifferentiated cells in the lateral zone of the gill filaments (Gros et al., 1997; Bright and Sorgo, 2003). From an evolutionary and developmental point of view, these data provide insights into the cell type or germ layer the bacteriocytes are derived from in those early lineages. However, in insects even with one of the best-researched models—the aphid and its associated endosymbiont *Buchnera*—the mechanisms, cell types, or germ layer that the bacteriocytes arise from remain unknown (Braendle et al., 2003; Simonet et al., 2018; Banfill et al., 2020; Davis et al., 2021). At the genetic level, it is suspected that the transcription factors *Ubx*, and *abdA* are participating in the formation of bacteriocytes in the aphid—*Buchnera* endosymbiosis system (Braendle et al., 2003). A closer examination of the hemipteran insect *N. plebeius* shows that *Ubx* and *abdA* genes normally involved in defining abdominal segments, seem to have been additionally co-opted to induce novel cells to form the bacteriocytes (Matsuura et al., 2015). Interestingly, in a different lineage, the *Camponotus* and closely related ants, the endosymbiont *Blochmannia*, homologs of the same genes appear to be involved in bacteriocyte development (Rafiqi et al., 2020). The bacterial endosymbiont *Blochmannia* is found in the ovaries of adult ants and the posterior pole of mature oocytes. When the oocytes transition to freshly laid eggs, *Blochmannia* is found at the posterior pole (Blochmann, 1882; Buchner, 1965; Sauer et al., 2000; Kupper et al., 2016; Ramalho et al., 2018). When the syncytial embryos form cellular boundaries, the bacteria get enveloped in bacteriocytes, which migrate to the middle of the yolk and become part of the midgut epithelium (Rafiqi et al., 2020). In some species of genus *Camponotus* an intriguing novel cell type called the "germline capsule," evolved to house a seed population of *Blochmannia.* This sub-population migrates to the germ cells and is transmitted to the next generation through the gonads (Rafiqi et al., 2020). The transition to obligate endosymbiosis therefore involves multiple changes in the host organisms that involve genetic and anatomical novelties that enhance the efficiency of the endosymbiotic association. Further exploration of the genetic signatures of these novel cells or structures in comparison to other neighboring or ontogenetically similar structures in these organisms will shed light on whether these evolved from pre-existing structures and genes or arose independently.

CONCLUSION

This review is an attempt to draw the framework of the study of developmental integration in endosymbiosis taking insects as a model. Because of their widespread occurrence, the integration of endosymbionts into a novel association is crucial for comprehending the evolution of life on Earth. The mechanism of establishment of endosymbiosis and its continuation through generations is in the early stages of exploration. Even though endosymbiotic associations have been known for a long time, it is important to point out that many of them were not understood at the molecular level. Moreover, there has been exceeding emphasis on the proximal interdependence between the endosymbiont and the host without regard to the developmental perspective. Recently, one of the biggest surprises in the studies of endosymbiosis has been that development not only affects the establishment, maintenance, and transmission of the endosymbiont but the endosymbiont also affects the developmental pattern formation of the host. Here, we propose that the developmental process plays an active role in coordinating and establishing endosymbiotic associations, incorporating inputs from the genome, and ecological interactions to efficiently engage the endosymbiont within the host. Subsequently, the organisms undergo changes that make them irreversibly interdependent. An interactive scheme encompassing and understanding the intersection of development with nutritional, metabolic, immune-related, genetic, or ecological aspects of these associations will reveal a broader and potentially more accurate picture. Future research on the key role of development integration in endosymbiosis will shed light upon the way endosymbiosis is established and maintained. More individual examples from the living world would be required to substantiate these ideas about the effect of development on endosymbiosis.

AUTHOR CONTRIBUTIONS

AMR conceived the idea and wrote the manuscript. PGP, NSM, ZÖD, BÇ-A, MEA, FZÇ, and AR wrote the manuscript. All authors contributed to the article and approved the submitted version.

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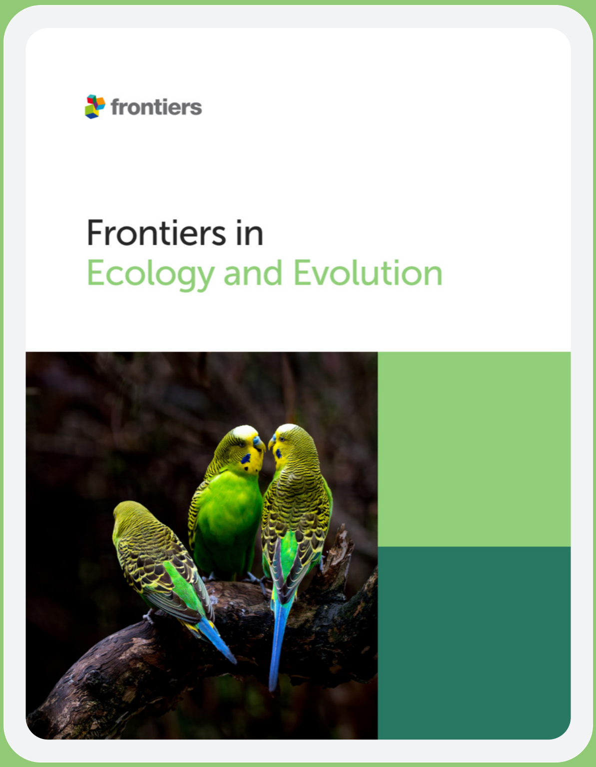

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