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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

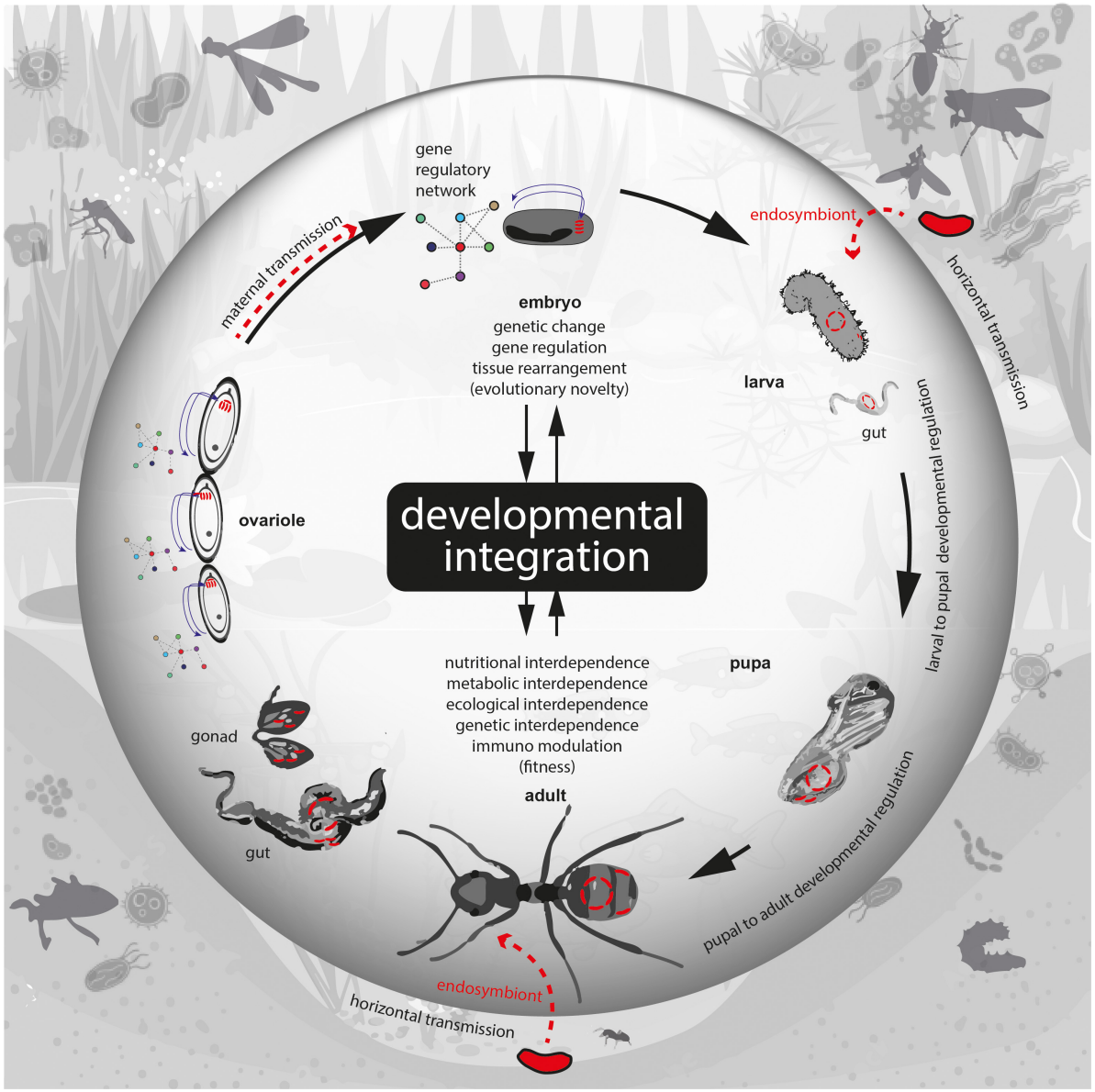

FIGURE 1 | Illustration showing developmental integration in the process of maintenance of endosymbiotic association between a bacterial endosymbiont and an ant host as an example. Bacteria are shown in red and bacterial cell walls in black. Different developmental stages are shown representing the lifecycle 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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