Eco-Evo-Devo: developmental symbiosis and developmental plasticity as evolutionary agents

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Abstract | The integration of research from developmental biology and ecology into evolutionary theory has given rise to a relatively new field, ecological evolutionary developmental biology (Eco-Evo-Devo). This field integrates and organizes concepts such as developmental symbiosis, developmental plasticity, genetic accommodation, extragenic inheritance and niche construction. This Review highlights the roles that developmental symbiosis and developmental plasticity have in evolution. Developmental symbiosis can generate particular organs, can produce selectable genetic variation for the entire animal, can provide mechanisms for reproductive isolation, and may have facilitated evolutionary transitions. Developmental plasticity is crucial for generating novel phenotypes, facilitating evolutionary transitions and altered ecosystem dynamics, and promoting adaptive variation through genetic accommodation and niche construction. In emphasizing such non-genomic mechanisms of selectable and heritable variation, Eco-Evo-Devo presents a new layer of evolutionary synthesis.

Evolutionary biology today tries to explain a natural world that appears remarkably different from the nature of the past century. It is a dynamic world, where symbiosis and phenotypic plasticity are the rules, not the exceptions. High-throughput sequencing has uncovered a world of complex interactions between developing organisms and the biotic and abiotic components of their environments. This newfound awareness of the dependency of phenotypes on other species and environmental conditions presents additional layers of complexity for evolutionary theory and raises many questions that are being addressed by new research programmes. The field of ecological evolutionary developmental biology (Eco-Evo-Devo) attempts to study and model this new view of nature by organizing concepts such as developmental symbiosis and developmental plasticity into evolutionary theory[1,2].

Developmental symbiosis is the concept that organisms are constructed, in part, by the interactions that occur between the host and its persistent symbiotic microorganisms. Although once thought to be exceptions to normal development, such developmental symbioses seem to be ubiquitous among plants and animals[1,3–5]. Recent studies document that developmentally active symbionts offer selectable genetic variation for the entire animal, and that they provide mechanisms for the reproductive isolation that can potentiate speciation. For instance, in the parthenogenetic aphid *Acyrthosiphon pisum*, the phenotypes of body colour[6], resistance to parasitoid infection[7] and thermotolerance[8,9] are all transmissible through the alleles of their symbiotic bacteria. In *Drosophila melanogaster*[10] and the wasp *Nasonia* spp.[11], different bacterial symbionts can generate reproductive isolation.

Developmental plasticity — the ability of larval or embryonic organisms to react to environmental input with a change in form, physiology or behaviour[12] — is also ubiquitous. A single genome can generate different phenotypes depending on environmental cues. This means that the environment is not merely a selective agent; it also shapes the production of phenotypes. Such developmental plasticity can be critical in evolution. First, such plasticity can provide the phenotypic ranges within which animals can accommodate to environmental challenges such as climate changes[13,14] As such, it is crucial in ecosystem modelling. Second, developmental plasticity can facilitate niche construction, the process whereby an organism actively alters its environment[15].

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Ecological evolutionary developmental biology
(Eco-Evo-Devo). The scientific programme that incorporates the rules governing the interactions between an organism's genes, development and environment into evolutionary theory.

Reproductive isolation
The phenomenon whereby members of two potentially interbreeding populations are prevented from producing viable or fertile hybrid offspring.

Holobionts
The eukaryotic organism (host) plus its persistent symbionts. The cow, for instance, is a combination of the mammalian body plus the symbionts, the enzymes of which allow it to digest grasses, and so on.

Microbiomes
The totality of microorganisms and their collective genetic material present in or on the body of a macroscopic host organism or in another environment.

And third, such developmental plasticity can generate environmentally induced phenotypes that might ultimately be assimilated into the genome to become inherited traits[12,16,17].

Both developmental symbiosis and developmental plasticity have been implicated in facilitating major transitions in evolution (BOX 1). Symbiosis may be responsible not only for the origin of eukaryotic cells[18], but also for the origin of new mammalian cell types[19,20], and for the origin of multicellularity itself[21,22]. Developmental plasticity has recently been suggested to have been critically important in the transition of fins into limbs[23]. In proposing such non-genomic mechanisms of selectable hereditary variation and in highlighting their importance, Eco-Evo-Devo presents a new layer of evolutionary synthesis.

In this Review, we focus on new research in the areas of developmental symbioses and developmental plasticity, and highlight the importance of these phenomena in evolution. We do not deal extensively with environmentally induced epiallelic inheritance (such as alleles differing in DNA methylation patterns[24–26]) or other aspects of Eco-Evo-Devo that are reviewed elsewhere[2,27].

Symbiosis and evolution

Life is sustained by symbioses between nitrogen-fixing rhizobial bacteria and legumes, sulphide-oxidizing bacteria and clams in tidal seagrass communities, algae and reef-building corals, and protective mycorrhizal or endophytic fungi and plants[27]. In addition to these grand symbioses are the nodes of symbiosis called organisms.

There are no germ-free animals in nature. Epithelia in contact with the environment are colonized by microbial communities, and all multicellular organisms must be considered as an association of the macroscopic host in synergistic interdependence with bacteria and numerous other microbial and eukaryotic species. These associations, which can be analysed, measured and sequenced, are referred to as holobionts[28] or metaorganisms[29]. The holobiont concept considers the dynamic microbial communities in or on animal cells and organs to be integral to the functionality of the host organism[4,5,30]. It is commonly agreed that metaorganisms are co-evolved species assemblages, but there is disagreement on how to characterize and quantify how natural selection operates on them. The increasing appreciation that animals exist only within a partnership with symbionts has led to three important realizations. First, to understand the physiology, evolution and development of a given species, we cannot study the species in isolation. Second, the health and fitness of animals, including humans, is fundamentally dependent on multiple organisms of several species. Last, the holobiont may be an important unit of evolutionary selection, whereby selection selects 'teams' containing many genomes and species.

Phylosymbiosis: microbiomes recapitulate host evolution. As symbionts provide intercellular signals that allow the development of the host organisms[4,5,29], we need to consider the evolution of the host together with its symbionts. Multicellular animals diverged from their protistan ancestors some 3 billion years after bacterial life originated[31]. Thus, relationships of protists with bacteria were likely to have already existed when animals evolved. Since then, animals seem to have intimately co-evolved with their specific sets of microorganisms, such that even closely related animal species reared on the same diet maintain unique microbiomes.

For instance, in wasps the composition of the bacterial communities in the gut differs in parallel with the phylogenetic relationships between the distinct host species. This co-evolution of hosts with their microbial communities has been termed phylosymbiosis[11]. Likewise, three species of Hydra maintain their specific bacterial symbionts even when cultivated together[32]. Similar patterns of phylosymbiosis are also evident in primates[33]. The animal microbiome has a phylosymbiotic signature that is structured by the host genome and recapitulates the ancestry of the host's evolution across lineages and species. Animals, therefore, have co-evolved with sets of specific microbes.

How a newborn animal acquires and assembles the specific set of microorganisms that it needs to survive, while avoiding and eliminating the microorganisms that might harm it is not well understood (BOX 2) but is currently the subject of intensive research.

Developmental symbiosis. More than just the product of co-evolution, the holobiont is a harmonized product of co-development. In numerous animals, symbiotic interactions are essential to development. For example, bacterial symbionts are essential for the metamorphosis

Box 1 | **Contribution of developmental symbiosis and developmental plasticity to animal evolution**

Developmental symbiosis and developmental plasticity contribute to evolution in numerous ways:

- Symbionts help to generate organs and to maintain species-specific interactions with their animal hosts
- Symbionts provide selectable variation through their presence or through having particular alleles
- Symbionts can generate the conditions for reproductive isolation
- Symbionts may have promoted major evolutionary transitions such as multicellularity
- Plasticity allows the integration of the organism into its environment, changing development to account for predators, conspecifics, diet and temperature
- Plasticity provides the raw material for genetic accommodation and adaptation to new environments
- Plasticity provides the raw material for niche construction
- Plasticity provides resources for adapting to stresses, including global climate change

Box 2 | **Microbial colonization of the newborn**

The ability of the human fetus to determine which bacteria stay and which ones must be excluded is still not understood[138]. Although there is evidence that some microorganisms are already present in the amnion[139,140], the major portion of the bacterial population of the mammalian gut is usually acquired as the fetus passes through the birth canal. These bacteria seem to be important, as babies born through caesarean section have an altered bacterial colonization pattern early in life compared with vaginally delivered babies[141,142]. Furthermore, babies delivered by caesarean section have a lower microbial diversity, delayed colonization with important microorganisms (such as Bacteroides) and reduced lymphocyte responses[143,144].

Hydra has been used as a model organism to investigate the colonization of the newborn animal. Microbial colonization of newly hatched *Hydra* embryos followed by 454 sequencing indicated that the colonization rate depended on local environmental or host-derived factors as well as interactions between individual bacteria[59].

The ability of any metazoan host to recognize bacteria is essential in determining whether they are allowed to colonize or are rejected, and context is crucial. The process of colonization seems to involve many of the same host factors that are usually involved in attacking bacteria (for example, Toll-like receptors and immunoglobulins), but symbiotic bacteria seem to have certain compounds on their surface that turn this recognition into acceptance rather than attack[56,145]. The mechanism by which this occurs is one of the fundamental questions in this new field.

of many invertebrates[34,35], for the formation of ovaries by the wasp *Asobara*[36] and for the germination of orchids[37]. Moreover, the anterior–posterior axis of the nematode *Brugia malayi* is generated with the help of *Wolbachia* bacteria, and if these bacteria are eliminated from the egg, the anterior–posterior polarity fails to develop properly[38]. These are just some examples, and it is possible that all animals form some of their organs through symbiosis.

The intestines of germ-free mice can initiate, but not complete, their differentiation, which suggests that bacteria also provide developmental signals to the intestinal epithelia and that the presence of microbial symbionts (or microbiota) is required for complete gut development in mice[39–41]. Mammals are not the only animals that depend on microbial symbionts to complete their gut development; in zebrafish, the gut microbiota uses the β-catenin signalling pathway to initiate cell division in the intestinal stem cells[42], and in the absence of a gut microbiota, zebrafish have smaller and less functional intestines, with a paucity of enteroendocrine and goblet cells. All of these defects can be reversed by the introduction of bacteria later in the zebrafish's development[43]. Notably, gene expression profiles comparing germ-free mice and zebrafish to their normally raised controls reveal remarkable parallels in their transcriptional responses to their gut microbiota, with especially significant changes in the expression of those genes involved in cell proliferation, nutrient utilization and immune function[44]. Thus, animals seem to have a conserved programme of interactions with the symbiotic microorganisms with which they have co-evolved, which are required for the completion of gut differentiation.

Pioneering work spearheaded by Margaret McFall-Ngai and Edward Ruby[45] (FIG. 1) has shown that the morphogenesis of the light organ of the Hawaiian bobtail squid *Euprymna scolopes* is actively induced by *Vibrio fischeri*, a bacterium that forms part of the complex seawater microbial community, and that the light organ fails to mature in squids raised without *V. fischeri*[45]. Transcriptomic analyses[46,47] revealed that when *V. fischeri* cells associate with the host along its superficial epithelium, the host recognizes and responds to the presence of these bacteria by expressing a chitinase that primes the bacteria to migrate by chemotaxis up a chitobiose gradient into host tissues. The squid–*V. fischeri* symbiotic system orchestrates a profound 'winnowing' from the thousands of bacterial species interacting with the surface of the light organ to the presence of just one or two strains of *V. fischeri* in the deep organ's crypts a couple of microns away. Once in the crypts, *V. fischeri* cells generate the light organ and, through their cell wall peptidoglycans and lipids, induce the loss of the superficial ciliated fields that had facilitated their colonization[48].

Symbiotic bacteria also shape the complex immune system of vertebrates. Compared with conventionally colonized mice, germ-free animals have serious immune system defects, including fewer lymphocytes and less active intestinal macrophages, as well as reduced vascularity, digestive enzyme activity, muscle wall thickness, cytokine production and serum immunoglobulin levels[49]. Unsurprisingly, these germ-free animals are more susceptible to infections than their conventional counterparts. Reconstituting germ-free mice with normal intestinal microbiota is sufficient to restore their mucosal immune system[50], and this seems to be the result of changes in the T cell populations[51]. This effect is mediated by a surface molecule of the bacterium *Bacteroides fragilis*, capsular polysaccharide A (PSA), which affects the development of systemic T cell responses and thereby influences the normal development and function of the mucosal immune system in colonized mice[52,53]. Changing the population of microorganisms in the newborn macaque (by changing its diet) dramatically alters the T cell populations[54]. Evidence for extensive inter-kingdom communication also comes from the observation that *Bifidobacterium breve*, a beneficial bacterium in the human gut, prevents intestinal inflammation by activating interleukin-10-producing regulatory T cells in the gut[55,56].

The gut microbiota also appears to direct development of the innate immune system by interacting with the factors that induce blood cell development, or haematopoiesis[57]. In germ-free mice both the number and function of specific myeloid cell progenitors are reduced, and recolonization of germ-free mice with a complex microbiota restores defects in the formation of myeloid cells and resistance to the pathogen *Listeria monocytogenes*. Thus, the gut microbiota may be instructing innate immune cell development by promoting haematopoiesis. These observations highlight the deep and general impact of bacterial symbionts on vertebrate immunity (BOX 3). However, the reciprocal is also true; the immune system of invertebrates and vertebrates also shapes the microbiota.

The mechanisms that recruit and organize the specific microbial colonies are the focus of much current research, and it is evident that forces exerted by both the

Germ-free mice
Mice bred in sterile facilities with no contact with microorganisms.

Chemotaxis
The movement of an entity such as a cell along a gradient of chemical concentration towards the source of the chemical.

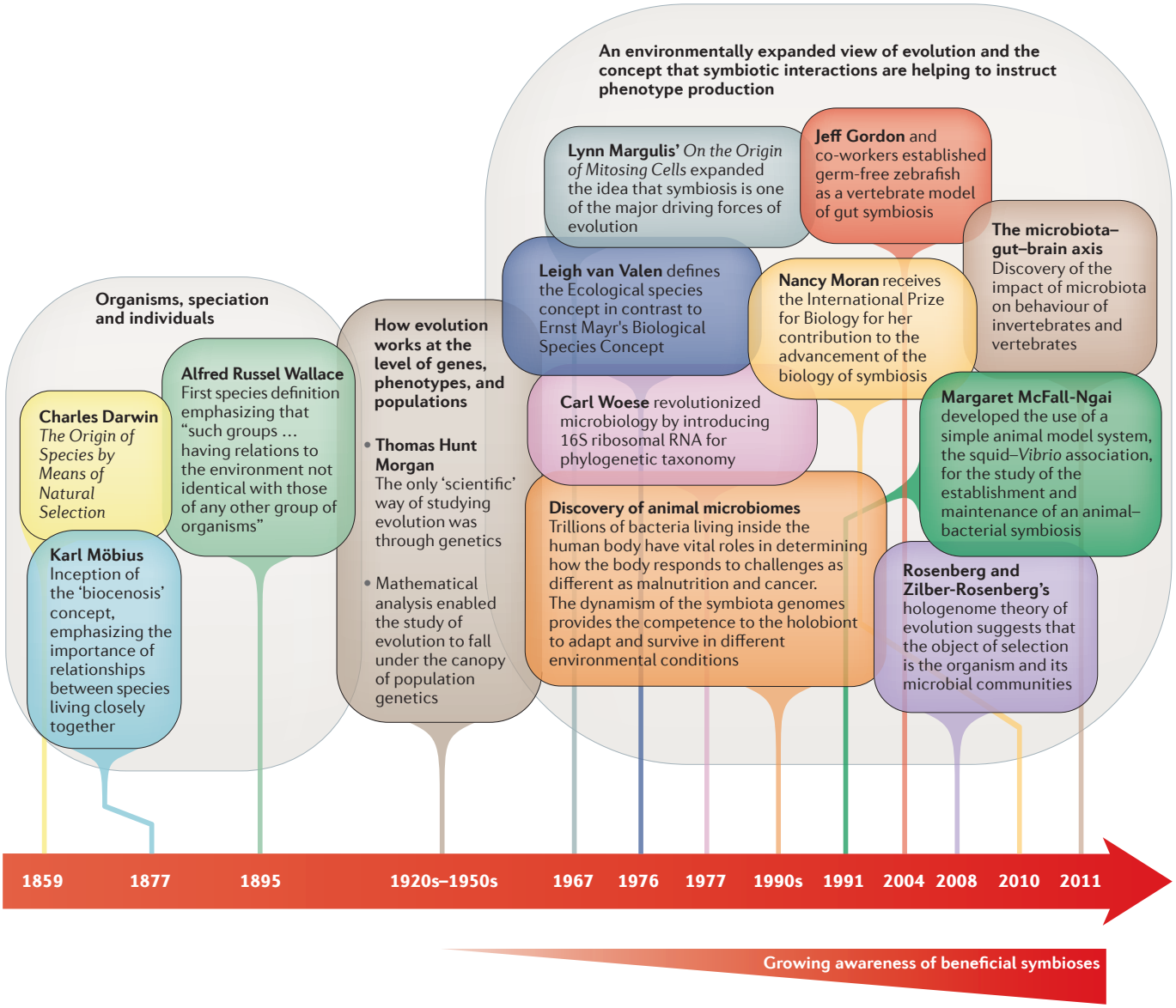

Figure 1 | **Milestones towards a new vision for the central importance of symbiotic interactions as being fundamental to all aspects of animal biology.** This vision is especially important in evolution, if phenotype is seen to be a product of the animal genome, the symbionts (and their genomes), and the abiotic environment.

host and the external environment help to mould these ecosystems[58,59]. Recent studies show that host factors, such as antimicrobial peptides (AMPs) and other components of the innate immune system, are extremely important in selecting a specific microbiota[32,59]. AMPs are prominent effector molecules of the innate immune system in both vertebrates and invertebrates, and they act by disrupting the structure or function of microbial cell membranes, thereby killing the microorganism[60,61]. Research in *Hydra* spp. and other invertebrates is now refining this view, by showing that AMPs can shape the species-specificity of the microbiota[61]. Similarly, mice that are either deficient in the functional AMPs known as α-defensins or that overexpress human α-defensin 5 show substantial α-defensin-dependent changes in microbial community composition[62]. Furthermore, patients with reduced immune function (due to different primary immune deficiencies) show significant differences in bacteria and fungi on their skin, compared with healthy individuals. This suggests that, as in invertebrates, the mammalian immune system constrains and potentially selects the species of bacteria and fungi that can inhabit the skin[63].

Thus, the innate immune system may have evolved not only for defence, but also because of the need to recognize complex communities of beneficial microorganisms and to maintain homeostatic relationships with them[64,65]. In addition, recent observations support the view[64] that microbial communities may have promoted the need for adaptive immunity in vertebrates. A study

Box 3 | **Microorganisms are integral components of the immune system throughout the animal kingdom**

As early as 1955 (REF. 146), it was shown that mice with an intact endogenous gut microbiota require 100,000 times higher inocula to establish *Salmonella enterica* infection than mice with a diminished microbial population due to streptomycin. This phenomenon is known as colonization resistance[147].

There have since been numerous studies identifying the integral role of commensal microorganisms in the development of animal immunity. Experiments using a gnotobiotic *Hydra* model[148] recently demonstrated that in the absence of commensal bacteria, the polyp life form is prone to fungal infection, and that restoring the specific microbiota prevents fungal infection. The *Hydra* study also found that multiple members of the microbiota act synergistically to confer resistance against the pathogenic fungus *Fusarium* sp.

Similarly, in amphibians many bacterial species have been found to inhibit the chytrid fungus *Batrachochytrium dendrobatidis*, which infects the outer layer of the skin[149,150]. Consistent with this, providing antifungal bacteria (*Janthinobacterium lividum*) to the skin of the mountain yellow-legged frog *Rana muscosa* prevents death from fungal infiltration[151].

In a mouse model of respiratory influenza, commensal bacteria provide a signal to the body that prepares the lungs to mount an immune response against viruses[152]. As a result, treating these mice with antibiotics (in the drinking water) leads to a significantly impaired immune response compared with the control group.

Systemic bacterial infections in mice activate a pathway that results in altered glycans on intestinal epithelial cells and induces rapid fucosylation of the intestinal epithelial cells. The availability of fucose is sensed by the gut microbiota, which is thought to promote host fitness by increasing tolerance to pathogens, possibly through the regulation of genes involved in virulence or quorum sensing[56,153].

in which T cells were transferred from normal mice into mice genetically lacking in adaptive immune cells demonstrated that skin bacteria are recognized by major populations of T cells, indicating a previously unrecognized role for T cells from skin-draining lymph nodes in controlling skin commensal bacteria[66]. Therefore, both innate and acquired immunity seem to be involved in maintaining the animal holobiont. The immune system has evolved as a form of ecosystem management that controls the composition, diversity and localization of the microbiota[65,67–69].

The normal development of the brain may also depend on microorganisms. The gut microbiota produces about 30% of the metabolites in mammalian circulation[5,70], including many neurotransmitters such as γ-aminobutyric acid (GABA), serotonin, histamine and dopamine[71]. Consistent with this, in germ-free mice, dopamine and glutamate receptor expression as well as serotonin levels are significantly altered in the circulation during brain development compared with conventional mice[72–74]. This establishes the gut microbiota–brain axis as an essential regulator of neurodevelopment, acting bidirectionally: the gut microbiota produces neuroactive compounds that influence the brain, and the brain acts on gut and immune functions that help to shape the gut's microbial population[75].

It is therefore not unexpected that microbial cues regulate animal behaviour. Indeed, the microbiota may be crucial in shaping host behaviours across many animal taxa, from fruitflies to humans and mice[74,76–79]. Germ-free mice exhibit behaviours of social avoidance, self-grooming, and other traits similar to those observed in disorders of neurodevelopment such as autism spectrum disorder (ASD)[80]. Moreover, the human gut microorganism *B. fragilis* can help to reverse some behavioural traits in a mouse model that displays features of ASD[81]. Microorganisms are being seen as integral to the processes of morphological and behavioural ontogenesis.

Gnotobiotic
A condition when the investigator knows all of the microorganisms in the host. Germ-free mice are often called gnotobiotic. Gnotobiotic animals are born in aseptic conditions and immediately transferred to an isolation area where all incoming air, food and water is sterilized.

The role of symbionts in evolutionary processes. In addition to playing a key part in the development of tissues and organs, symbionts have other crucial roles in evolution and development. Microbial symbionts form a second type of genetic inheritance, being acquired either through the egg or from the maternal environment[82,83]. Genetic variation in symbionts can provide phenotypic variation for the holobiont.

As mentioned above, symbiotic bacteria provide the pea aphid *A. pisum* with allelic variation that results in selectable traits (such as thermotolerance, colour and parasitoid resistance) that enable some holobionts to persist under different environmental conditions[6–8]. Thus, whether the holobiont has cryptic coloration, protection against parasitoid wasp infection or the ability to reproduce in hot weather depends not only on the host's genome, but also on the genomes of its symbionts. In such cases, the symbiotic bacterium is not necessary for the development of the holobiont, but its presence or absence (or the presence or absence of one of its alleles) can determine the holobiont phenotype. Recently, to better understand this symbiotic relationship, Nancy Moran and Yueli Yun[9] experimentally exchanged a heat-sensitive *Buchnera aphidicola* genotype for a heat-tolerant one in *A. pisum*. Intriguingly, aphids with the heat-tolerant *Buchnera* replacement showed a significant increase in their heat tolerance, which highlights not only the presence of a crosstalk between aphids and their new symbionts but also an effect of symbiont genotype on host ecology (FIG. 1).

The role of symbionts in speciation. The study of evolution is experiencing a significant convergence with studies of microbial symbiosis[84–86]. Reproductive isolation is essential for speciation, and recent evidence suggests that symbiotic microorganisms may facilitate such isolation. Experiments to study the basis of reproductive isolation in three related wasp species[11] have identified the gut microbiota as a cause of hybrid lethality. The wasp species

Box 4 | **The roles of developmental plasticity in evolution**

Developmental plasticity promotes niche construction, whereby the phenotypes of organisms are plastic in response to the developmental environment that they, themselves, have altered. For example, if *Wolbachia* bacteria find themselves within the cells of a genetically male pillbug (*Armadillidium vulgare*), they will convert the male into a female so that they might be transmitted to the next generation[154]. Similarly, the larvae of the goldenrod gall fly (*Eurosta solidaginis*) secrete factors that induce the stem of the goldenrod plant to form the gall where the fly can reside and eat[155]. Niche construction is evolutionarily relevant because it alters the selective environment of the organism eliciting the plastic response and, moreover, some genotypes are more likely than others to experience certain selective environments[123,156] (as there is genetic variation in niche-constructing abilities).

Developmental plasticity forms the basis of extragenetic inheritance, one mechanism of which is the ability of parents (or parentally modified environments) to influence the developmental environment in which their offspring develop, and thereby influence the phenotype of their offspring. An increasing number of studies have demonstrated the epigenetic mechanisms by which this transmission of information can occur, such as through hormones and microRNAs, as well as DNA methylation and histone modifications. In some instances, epialleles responsible for transgenerational inheritance of acquired traits can persist for hundreds of generations[24–26,157,158]. Whereas the modern synthesis proposes that genetic mutations are sufficient to generate the heritable phenotypic variation on which natural selection acts, evolutionary ecological developmental biology contends that extragenetic inheritance of phenotypic variation may also have an important role in evolutionary change.

Developmental plasticity is at the core of plasticity-driven adaptation, including phenotypic accommodation (whereby plasticity facilitates adaptive phenotypic adjustments without the need for genetic change) and, subsequently, genetic accommodation (whereby natural selection acts on the regulation of environmentally sensitive phenotypes). This selection on genetic variation underlying the regulation of plasticity (in other words, variation caused by genotype-by-environment interactions) can lead to the evolution of canalized, environmentally insensitive phenotypes or dramatic, threshold polyphenisms. This is discussed further in the main text.

Nasonia giraulti and *Nasonia longicornism* have a similar range of gut bacteria and can produce healthy hybrid offspring, but when either wasp mates with the more distantly related *Nasonia vitripennis*, which has a different gut microbiota, their hybrid offspring die. By contrast, when the hybrid offspring of *N. vitripennis* and each of the other wasp species are raised in a germ-free environment, their hybrid offspring survive. Furthermore, when germ-free offspring of *N.vitripennis* are inoculated with the gut microorganisms from either of the two other parent species, they die. Thus, a mismatch between the hybrid wasps and their inherited gut microbiota seems to be lethal. This suggests a possible evolutionary process whereby populations become increasingly reproductively isolated through the divergence of their microbiomes, and may lead to the formation of new species.

Another example of symbiont-induced reproductive isolation is a mating preference exhibited by *D. melanogaster*. *D. melanogaster* shows a strong mating preference for individuals that were reared on the same diet as they were[10]. This mating preference is abolished after antibiotic treatment and restored after inoculation of treated flies with microorganisms from the dietary media, indicating that mate choice is determined by microorganisms rather than diet. The changes in mating preference were linked to the presence of one bacterium, *Lactobacillus plantarum*, which was found to alter the cuticular hydrocarbons that form part of the mating pheromones of the adult fly.

Symbiosis is also thought to have been involved in some of the major transitions in the history of life. The endosymbiotic theory of eukaryotic cell formation holds that the origin of eukaryotic life began through the merging of Archaea and bacterial cells and genes[18]. Similarly, animal multicellularity might have emerged from the symbiosis of a choanoflagellate protist with a particular bacterial partner. Choanoflagellates, considered to be the sister group of animals, can produce unicellular or colonial morphotypes in response to certain bacteria[21,22]. In the choanoflagellate *Salpingoeca rosetta* a sulphonolipid signalling molecule produced by a bacterium from the Bacteroidetes group[87] is sufficient to trigger multicellular colony formation. These multicellular aggregates have cytoplasmic connections between their cells as well as a new extracellular matrix around them; they are not loose colonies but appear to be multicellular organisms. Another evolutionary transition, the origin of placental mammals, may have been permitted and promoted by symbiosis, namely the incorporation of retroviruses from other organisms. These retroviruses, which contain their own enhancer elements, seem to have allowed the rewiring of cell circuitry to produce the progesterone-responsive uterine decidual cell[20] as well as the syncytin fusion proteins of the mammalian placenta[88].

Thus, symbionts are crucial for normal development and evolution. They help to generate organs, they can produce selectable variant phenotypes, they can create the conditions for reproductive isolation, and they may be the facilitators of evolutionary transitions. Symbiotic relationships are the signature of life on earth, and evolutionary biology has to include developmental symbiosis as a major component. "Biology has entered a new era with the capacity to understand that an organism's genetics and fitness are inclusive of its microbiome." (REF. 89)

Developmental plasticity and evolution

Developmental plasticity has been shown to be a major driver of adaptive change. Developing organisms can alter their morphology, physiology and behaviours in response to numerous environmental conditions, including the presence of predators, conspecifics, specific foods, temperature, stress and crowding[27]. Developmental plasticity can either promote[12,90] or delay[90–92] adaptive evolution, and research towards understanding the causes and consequences of developmental plasticity has made key contributions to evolutionary theory (BOX 4).

However, there is still debate as to whether developmental plasticity is under-appreciated or well-integrated in the standard evolutionary theory[93]; we have to ask whether developmental plasticity, itself, has an empirical, conceptual and theoretical framework on par with that developed for the genetic paradigm. Fully incorporating developmental plasticity into evolutionary biology will take at least three steps: first, understanding the breadth and importance of plasticity in major evolutionary transitions; second, understanding the developmental mechanisms underlying plasticity[94]; and third, creating a theoretical framework in which the evolutionary outcomes of plasticity can be predicted for specific

Extragenetic inheritance
Mechanisms of inherited variation that are not derived from nucleic acid composition variants in the parent.

Epialleles
DNA sequences that are identical by nucleic acid composition but may differ in their secondary modifications such as DNA methylation, histone acetylation or methylation, or chromatin context. Also known as epimutations when they differ from wildtype.

Genotype-by-environment interactions
Processes wherein different genotypes respond to environmental variation in different ways.

Choanoflagellate
A group of unicellular and colonial flagellates that are thought to be the sister group of multicellular animals.

Box 5 | Cryptic genetic variation

Cryptic genetic variation — which produces no phenotypic difference under normal conditions but can be uncovered during periods of stress or environmental change — has been long known, but its widespread nature and importance to evolution have only recently been appreciated[103,159,160]. It was predicted that the unmasking and subsequent selection of existing, cryptic genetic variation in populations was responsible for the assimilation of traits[16,96]. Consistent with this hypothesis, genetic accommodation experiments showed that four alleles of the *Drosophila melanogaster* Ultrabithorax (*Ubx*) gene, which were crucial for the genetic assimilation of the ether-induced bithorax phenotype, were already present in the untreated *D. melanogaster* population[98]. Cryptic genetic variation is also thought to be responsible for the evolutionary transition to carnivory (that is, shrimp-eating) in the spadefoot toad tadpoles[161], and such variation might in general facilitate rapid evolutionary transitions to use new food sources.

The mechanisms for masking cryptic genetic variation remained speculative until experiments were carried out to investigate the effects of mutations in the *D. melanogaster* heat shock protein gene *Hsp83* and the inactivation of its protein product, HSP90 (REF. 162). When this gene was inactivated, pre-existing mutations in the population were exposed, and new phenotypes appeared. Moreover, the flies could be selected such that, within several generations, almost all expressed the mutant phenotype, and some of the flies had that phenotype even if they contained a functional *Hsp83*. In this way, HSP90 seems to be a capacitor for evolutionary change, allowing genetic changes to accumulate until environmental stress reveals their effects on phenotype.

The importance of cryptic genetic variation in natural populations is a largely unanswered question that awaits empirical studies. Still, all evidence from the handful of studies that we have suggests that cryptic genetic variation is widespread and can underlie substantial phenotypic variation. For instance, HSP90-buffered variation is so widespread through the plant species *Arabidopsis thaliana* that every quantitative trait is predicted to have at least one major component buffered by HSP90 [163,164]. Similarly, HSP90 was shown to mask cryptic genetic variation that affects the eye size in surface relatives of blind cavefish. Inhibiting HSP90 in cavefish increased the standard deviation of eye orbit size. Moreover, raising surface fish in cave-like situations taxed the HSP90 and allowed the cryptic variation to become expressed[165]. Finally, by selecting the smallest eyes among stressed fish, one could evolve a population that had eyes so small that they were not in the range of the parent population. Thus, HSP90 appears to be a major cause of the canalization of phenotypes, enabling the same wild-type phenotype to be displayed across a range of genetic and environmental conditions.

populations. Below, we look at recent advances towards accomplishing these goals and discuss the special importance of developmental plasticity in the context of global climate change.

Developmental plasticity and evolutionary transitions. According to the plasticity-driven model of evolution, developmental plasticity can initiate the generation of new traits that may improve an organism's viability under particular conditions. If there were heritable variation among members of a population in their ability to develop this newly favoured trait, then selection should favour those alleles or allele combinations that best stabilize, refine and extend the new trait's expression[12,16,95–97]. For instance, traits can subsequently become genetically assimilated in the lineage and robust against further environmental change or, in variable environments, become more environmentally responsive. This evolutionary process is referred to as genetic accommodation[12].

Genetic accommodation was demonstrated in the laboratory as early as 1953, when the crossveinless phenotype in *D. melanogaster* was induced by heat shock[16] and was then assimilated genetically in lineages by selective breeding. By the end of 14 generations, these experiments had produced lines of flies that developed the initially sensitive phenotype even at normal temperatures. Forty years later, similar selection experiments were conducted on flies that sometimes develop the bithorax phenotype in response to ether. Under selection, ether sensitivity increased, as did the prevalence of four specific mutant alleles of the Ultrabithorax (*Ubx*) gene in the bithorax complex[98].

More recently, selection experiments were carried out on plastic phenotypes using a variety of tobacco hornworm (*Manduca sexta*) that produces a gradation of black, pigmented cuticle at warmer temperatures. With selection, lineages were produced that were either liberated from environmental control (monophenic black lines that were not temperature sensitive) or that were plastic and responded to temperature in a threshold manner (lines exhibiting polyphenism). In the laboratory and in nature, the evolution of lineages that are more or less plastic relies on the exposure of regulatory genetic variation that is expressed in an environmentally dependent manner, sometimes referred to as cryptic genetic variation (BOX 5).

The process of genetic accommodation, so elegantly demonstrated in the laboratory (and also supported by modelling[99–101]), is likely to have been crucially important in the processes of natural adaptation and speciation. How does one test whether plasticity has played a role in the evolution of a specific trait? Although there is generally no way to resurrect ancestral species or populations (with the exception of some unique systems, for example, *Daphnia* spp.[102]), one powerful approach has been to test the ability of an extant species possessing ancestral traits to elicit a phenotypic response when challenged with environmental changes that mimic an evolutionarily relevant environmental transition. In many cases, these ancestral species and populations elicit phenotypic responses that mirror novel traits which are standard for derived lineages, thereby revealing a signature of genetic accommodation. For instance, one recent study investigated the Senegal bichir (*Polypterus senegalus*), a fish that possesses both primitive and derived traits with respect to the evolution of terrestrial locomotion[23]. Although this fish has the fins and body form that are characteristic of aquatic vertebrates, it can live on land and emulate terrestrial locomotion with its pectoral fins. The authors hypothesized that if plasticity facilitated the transition of limbed vertebrates from water to land, a species like *Polypterous senegalus* would display phenotypic responses to a terrestrial environment that parallel phenotypes observed in extant, terrestrial vertebrates. Indeed, they found that the behaviours of *Polypterus senegalus* that had been raised on land were more conducive to terrestrial motion relative to those that had been raised in the water; land-raised individuals moved more quickly across land, slipped less often and had less erratic motions. Most importantly, these behavioural differences instigated alterations in skeletal growth, producing new bone morphologies that mirrored the evolutionary changes made by stem tetrapods during the Devonian period. This study demonstrated that plasticity can generate

Genetic assimilation
A subset of genetic accommodation, whereby a trait induced by the environment becomes part of the genetic repertoire of the organism.

Bithorax
When the third thoracic segment of a fly becomes a repeat of the second thoracic segment, creating two sets of wings.

Polyphenism
The phenomenon when the same genotype can give rise to two or more distinct functional phenotypes.

behavioural and anatomical changes in one generation that are adaptive for a major environmental transition and could potentially fuel a macroevolutionary change.

Although the Senegal bichir study demonstrated plastic changes that are considered macroevolutionary in nature, the comparisons were drawn between lineages that are phylogenetically disparate and have therefore evolved independently for long periods of time. Thus, the degree of plasticity in each lineage could also have independently evolved. Fortunately, the same types of comparisons can be conducted between closely related species, and even populations, to determine whether derived traits mirror ancestral plastic responses to environmental change. For instance, studies[103] have shown that a species of spadefoot toad that possesses an ancestral feeding strategy (detritivory) responded plastically when fed a diet that it would not typically consume in nature (shrimp) by developing trophic traits that were more similar to lineages of spadefoots with novel, carnivorous feeding strategies. Importantly, this study demonstrated heritable variation for plasticity, and thus potential for the evolution of the regulation of plasticity. Currently, there is a wealth of similar studies from several other organisms — spadefoot toads[104], tiger snake[105], zooplankton[106] and stickleback fish[107] — that have demonstrated the evolution of adaptive plastic responses within short periods of time.

To summarize, these comparative studies have corroborated the notion that novel, adaptive, and heritable phenotypes can be elicited through developmental plasticity, and that processes such as genetic accommodation may be extremely important in evolutionary transitions.

Developmental plasticity at a mechanistic level. Although developmental plasticity has long been implicated as a major force in adaptation, speciation and macroevolutionary change, we are just now beginning to understand plasticity at a mechanistic level. It is important to understand plasticity at a mechanistic level to predict the potential (or constraints on potential) influences of plasticity on evolutionary outcomes. For instance, most evolutionary models that incorporate developmental plasticity make assumptions about the genetic architecture of plastic traits: how many genes are involved, the sizes of their effects, and whether they have discrete or continuous expression patterns[108]. The twenty-first century has seen the evolution of our approaches to understanding the developmental genetic underpinnings of plasticity from the purely conceptual[109] to molecular analyses at the level of specific genetic pathways, genome-wide assays, and functional analyses of specific developmental switch genes. Some of these recent accomplishments are described below.

One approach to understanding the developmental genetic basis of environmentally sensitive traits has been to examine the expression patterns of genes that are orthologous to those that underlie constitutively expressed traits in model organisms. For instance, one study[110] used knowledge of wing-patterning gene networks in *D. melanogaster* (which always develops wings) to investigate the behaviour of this pathway in *Pheidole* ants (which develop wings in an environmentally dependent manner). If *Pheidole* embryos are exposed to appropriate photoperiodic and temperature cues, they experience a pulse of juvenile hormone that causes them to develop into queens. If not, they develop as workers, which may further develop into soldiers if they are exposed to the appropriate diet. The investigators found that the expression of the genetic network components underlying wing patterning in *D. melanogaster* is conserved in winged, reproductive castes of *Pheidole* ants, but is interrupted in sterile, wingless castes (soldiers and workers). Furthermore, these disruptions happened at different points in the wing-patterning network depending on what species, wingless caste (soldier or worker) and set of wings (fore or hind) was being considered. These types of investigations using candidate pathways have revealed that the evolution of plastic traits is probably more labile among tissues (reducing potential pleiotropy among plastic traits) and species than previously speculated. Indeed, more recent genome-wide studies have also shown that different genes may mediate plasticity in different tissues (even in response to the same environmental cue[111]). This suggests that tissues and body regions can independently evolve plastic responses that comprise an integrated phenotypic response.

Although the investigation of candidate pathways has generated a solid mechanistic foundation for our understanding of environmentally sensitive traits, such analyses do not easily allow the discovery of potentially important but unintuitive pathways and networks. However, research using transcriptomic approaches (such as next-generation sequencing and microarray analyses) has removed such constraints. For instance, a microarray-based study was conducted on *Onthophagus* beetles, which exhibit nutrition-dependent polyphenism with respect to head horns (only well-fed, large males develop head horns[112]). This study revealed that the sex-determination gene doublesex (*dsx*) was expressed specifically in the horn tissue of large males. A subsequent study confirmed a functional role for *dsx* in *Onthophagus* head horn plasticity: RNAi-mediated knockdown of *dsx* reduced horn growth in a nutrition-dependent manner, and the effects were greatest in large males[113]. Importantly, the study of *dsx* had been previously confined to its influence on sexual dimorphisms; this approach demonstrated that *dsx*-mediated regulation of sex-specific traits has been coopted evolutionarily for the regulation of nutrition-specific traits[114].

An additional, unbiased and potentially powerful approach towards discovering the genetic mechanisms underlying plasticity is to employ forward genetics. One recent study[115] used this method to examine a developmental switch underlying resource polyphenism in nematodes. Under low population density conditions, the nematode *Pristionchus pacificus* develops simple mouthparts that are specialized for an ancestral diet of bacteria. However, under population-dense conditions, *P. pacificus* larvae develop into adults with complex teeth that can cut through cuticle and that enable the worm to consume other nematodes. Using a forward genetic screen, the

authors identified a single gene — the sulphatase-encoding *eud1* — as responsible for the development of the carnivorous morph. This regulatory developmental switch apparently evolved as the result of gene duplications in a rapidly diversifying clade the component lineages of which vary in the degree they express the polyphenism, with some lineages being canalized for either morph[116]. The tractability of this model system for forward and reverse genetics, along with its diversity in plastic forms, will make it particularly amenable to understanding the genetic changes (for example, gene duplications, protein modifications or regulatory sequence modifications) that contribute to the regulatory evolution of developmental plasticity. The nature of the genetic mechanisms underlying the genetic accommodation of such environmentally induced traits is still largely unknown (BOX 5).

Conceptual framework for developmental plasticity. It is clear from the previous section (see also BOX 5) that we have made great strides in uncovering the mechanistic bases of developmental plasticity, and of the buffering systems that conceal developmental plasticity. However, for environmentally dependent variation to gain a place alongside genetic variation in evolutionary theory, it is necessary to generate a unified framework that incorporates plasticity into evolutionary change with testable predictions that are validated by empirical data from natural populations.

For some themes in developmental plasticity, this has been accomplished. For instance, conditionally expressed genes such as maternal effect genes (which are expressed only in females) are expected to experience relaxed selection because they are exposed to selection less often than genes that are expressed unconditionally[117,118], and therefore harbour more genetic variants. Such accumulated variation can drive evolutionary change, resulting in rapid divergence in conditionally expressed genes and their overlying traits between different lineages. The same applies for other sex-limited genes[119]. Similarly, the expression of environmentally dependent genes (and phenotypes) should result in higher genetic variation within, and divergence between, lineages, and this has recently been observed (for example, nutrition-dependent gene expression[120]).

Bodies of theory have been developed to incorporate other Eco-Evo-Devo themes (for example, gene–culture co-evolution, indirect genetic effects, maternal effects, transgenerational epigenetics and niche construction), and models have been developed that attempt to bring these theories under one umbrella[121]. What remains to be done is to generate creative approaches to collecting empirical data from natural populations to test predictions from these models. Although such predictions may be straightforward in laboratory populations, plasticity in natural populations is characterized by synergies, thresholds and local ecotypes, which might make outcomes (and even extrapolations from known systems) difficult to predict. However, such modelling has the potential to explain ecosystem dynamics and the complex relationships that exist between species[121].

One particular type of plasticity that deserves more attention with respect to conceptual and theoretical modelling is niche construction[15,122] (BOX 4), which is apparently ubiquitous among animals. Empirical laboratory experiments have been successful in demonstrating that niche construction evolution occurs, specifically by meeting the prediction that niche constructors should enjoy higher fitness in their evolved niche-constructed environments relative to their ancestral niche-constructed environments[123]. However, a framework for predicting when and how it may influence evolutionary trajectories is lacking. Thus, although niche construction may be widely acknowledged by the evolutionary community, this process has not been successfully incorporated into evolutionary theory. Creating a solid framework for the testing of evolutionary outcomes of niche-constructing populations will be a difficult undertaking for the following reasons: first, niche construction encompasses several phenomena that vary in how many individuals of a population they influence, how they influence the phenotypes of individuals and how heritable they are over generations; and second, individual differences in niche-constructing behaviours or traits can be influenced by genetic variation, plasticity, gene-by-environment interactions and epigenetic inheritance. Nonetheless, it will be important to evolutionary theory to model such outcomes, because niche construction can influence the expression of, and genetic variance in, whole suites of characteristics that are altered by the niche-constructing trait or behaviour[124], and can influence the strength of selection experienced by populations, thereby influencing the direction and magnitude of evolutionary change. Such analyses of niche construction provide an important task for Eco-Evo-Devo.

Developmental plasticity and global climate change. We are living in a time characterized by some of the most rapid climate changes in the world's history, including higher mean temperatures throughout much of the world[125,126]. These climatic changes can disturb the interactions that occur between developing organisms and their environments.

Many animals have a developmental stage that can only be initiated or completed within a strict range of temperatures. Egg-laying species, especially tropical turtles, have embryonic stages that have much lower thermal tolerances than their adult stages, and increased temperatures threaten the survival of turtles in the eastern Pacific Ocean[127,128]. Heat waves produce morphological abnormalities in pond turtles, and these female turtles are less fit than the unstressed females[129]. Likewise, animals with temperature-dependent sex determination (such as turtles) will be negatively affected by climate change[130,131]. For many marine turtles, the sex ratio is already skewed towards producing females, and in some rookeries of the endangered green turtle (*Chelonia mydas*), the sex ratio is 95:5 female to male. As females are produced at higher temperatures, the predicted increases in global temperature are expected to acerbate this trend, spelling disaster with respect to the genetic diversity of the population by reducing the effective population size.

Modern synthesis
Also called the neo-Darwin synthesis, this model of evolution reconciles natural selection with Mendelian genetics.

Phenology — the timing of life cycle events in plants and animals, for example eclosion of pupae into adult insects — can also be disrupted by climate change. Phenology is crucial to many plant and insect species, where the flowering of the plant must occur at the same time as the eclosion of the insect pollinators. If the opening of the flower and the adult stage of the pollinator fail to occur simultaneously, both species might become extinct[132,133]. Long-term observations of plant–pollinator interactions strongly suggest that changes in phenology can cause the extinction of specialist pollinators (such as those that can only pollinate a single species). By contrast, plant species with several pollinators seem to be protected from extinction[134].

There are two major ways for commensal symbioses to survive climate-induced asynchrony in their life cycles: genetic diversity in the population, such that there are different alleles conferring different timings, and plasticity, such that at least one of the interacting species can accommodate the other. And if plasticity itself is an inherited trait, then the two mechanisms can be intimately linked. Moreover, developmental plasticity can produce a phenotype that buys time for populations to evolve by mutation and recombination, while keeping fitness stable. However, the study of plasticity and its limits is still in its infancy, and we know little about how well natural populations can withstand climate change[117].

Conclusions

Leigh van Valen[135] famously said: "A plausible argument could be made that evolution is the control of development by ecology. Oddly, neither area has figured importantly in evolutionary theory since Darwin, who contributed much to each." (FIG. 1). Developmental symbioses and developmental plasticity are two of the phenomena bringing development and ecology back into evolutionary theory, in ways that interact with and extend the modern synthesis. These are part of a larger programme, known as ecological evolutionary developmental biology[7,136] (which has also been called, with different emphases, the developmental synthesis (REF. 1) and the extended synthesis[137]). This programme seeks to fuse evolutionary theory with the rules governing the interactions between an organism's genes, development and environment.

The newly discovered, interactive, world of holobionts and instructive environments is a nature that is different from the biomes seen through the lens of the modern synthesis. Animals are not individuals by the traditional anatomical, physiological, immunological, genetic or developmental accounts. Rather, developmental symbiosis generates holobionts, organisms that are composed of numerous genetic lineages the interactions of which are crucial for the development and maintenance of the entire organism. Moreover, the environment is not merely a selective filter. Developmental plasticity transforms the environment into an active agent in shaping the phenotype. With these changes comes a shift in how we think evolution works. Natural selection may function at the level of the holobiont, genes can sometimes be considered followers, not leaders of phenotypic evolution, and developing organisms can modify their environments and then be modified by them. Documenting, comprehending, and understanding the ramifications of these phenomena are the areas of ecological evolutionary developmental biology.

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The authors declare no competing interests.