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Developmental symbiosis facilitates the multiple origins of herbivory

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Abstract

Developmental bias toward particular evolutionary trajectories can be facilitated through symbiosis. Organisms are holobionts, consisting of zygote-derived cells and a consortia of microbes, and the development, physiology, and immunity of animals are properties of complex interactions between the zygote-derived cells and microbial symbionts. Such symbionts can be agents of developmental plasticity, allowing an organism to develop in particular directions. This plasticity can lead to genetic assimilation either through the incorporation of microbial genes into host genome or through the direct maternal transmission of the microbes. Such plasticity can lead to niche construction, enabling the microbes to remodel host anatomy and/or physiology. In this article, I will focus on the ability of symbionts to bias development toward the evolution of herbivory. I will posit that the behavioral and morphological manifestations of herbivorous phenotypes must be preceded by the successful establishment of a community of symbiotic microbes that can digest cell walls and detoxify plant poisons. The ability of holobionts to digest plant materials can range from being a plastic trait, dependent on the transient incorporation of environmental microbes, to becoming

a heritable trait of the holobiont organism, transmitted through the maternal propagation of symbionts or their genes.

Key words: holobiont, herbivory, evolutionary bias, niche construction, genetic assimilation, EES

Research highlights:

The concept that developmental bias can influence evolution is fulfilled when one perceives the organism as a holobiont and views development as a multi-species process. For herbivory to originate, animals need to obtain the proteins needed to digest plant cell walls. This can occur through the acquisition of microbes through symbiosis or through the acquisition of such genes by lateral gene transmission from such herbivorous microbes. In many instances, the microbes construct niches in the developing organism.

I. Introduction

 The perception of biological individuality has changed dramatically over the past twenty years. Polymerase chain reaction and high-throughput RNA techniques have shown that our anatomical and physiological phenotypes come not only from the eukaryotic cells we inherit from the zygote, but also from the trillions of microbial cells that we inherit from the mother and acquire from the environment. An animal (or plant) plus its microbial communities is called a holobiont. Evidence suggests that most, if not all, animals develop as holobionts, consortia of symbiotic species. Complex holobionts such as ourselves are both organisms and biomes, collections of interacting ecosystems (*e.g.,* Lee and Mazmanian 2010; Ledón-Rettig et al., 2018) . These symbiotic microbes can be agents of developmental plasticity, allowing an organism to develop in particular directions. This plasticity can be adaptive, with the symbiont alleles providing instructions for specific trajectories of development. While we inherit only 22,000 different genes from our parents, we acquire over 8 million different genes by the colonization of our gut and other epithelial surfaces by bacteria (Gilbert et al., 2012; Funkhauser and Bordenstein, 2013; McFall-Ngai et al., 2013). Thus the microbial component of the holobiome has great potential to affect host phenotype, fitness, and perhaps other areas of development and evolution.

The holobiont is an anatomical, physiological, developmental, and immunological unit (Zilber-Rosenberg and Rosenberg, 2008; Gilbert et al., 2012, McFall-Ngai et al., 2013; Rosenberg and Zilber-Rosenberg, 2016; Roughgarden et al., 2017). The holobiont nature of animal anatomy and physiology is highlighted in several species where the microbes play critical roles in defining the organism. A cow is considered an herbivore, but without its community of cellulose-digesting enzymes within its specialized rumen stomach, it cannot digest plant material. In reef-building corals, the algal symbiont, *Symbiodinium*, enters into the ectoderm of its host (zoon) and transports over 90% of its photosynthetically derived carbon compounds to the host cells (Muscatine et al., 1984). In exchange, the coral gives those endosymbionts critical nutrients and a safe, sunlit habitat in an otherwise nutrient-poor habitat (Roth, 2014). Remarkably, the immune system of an animal turns out to be a holobiont property, as the colonization of the animal is often seen to be facilitated by the immune system, and the microbes help induce the host immune tissue (Tauber, 2009; Eberl, 2010; Pradeu, 2012).

These properties of holobionts have important evolutionary implications. The "hologenome concept of evolution" considers the holobiont with its hologenome (the collective genomes of the microbes as well as the zygote-derived cells) as a level of selection in evolution (Zilber-Rosenberg and Rosenberg, 2008; Roughgarden et al., 2017; Osmanovic et al., 2018; Roughgarden 2019). Holobionts meet the criteria for being considered evolutionary agents, as they are interactors, replicators/reproducers, and manifestors of adaptation (Roughgarden et al., 2017). In addition to their ability to provide heritable and selectable variation (see below), symbionts have also been suggested as means to facilitate both pre-zygotic and post-zygotic reproductive isolation (Sharon et al., 2010; Brucker and Bordenstein, 2013; Rosenberg et al., 2018).

In this paper, I aim to show that symbionts can facilitate a major evolutionary innovation--herbivory. First, symbionts will be shown to be agents of developmental plasticity. Then, given this ability to alter phenotypes, I will show

that symbionts can induce niche construction and be genetically assimilated into the heritable repertoire of the organism. Last, we will see that these properties-- plasticity, niche construction, and genetic assimilation--facilitate the origins of herbivory in insects and vertebrates. In this way, sympoiesis, the development of the holobiont, might be critical in producing evolutionary bias, the symbionts scaffolding the anatomical and physiological underpinnings of the herbivore radiations in vertebrates and invertebrate lineages.

II. Symbiosis as a mechanism for developmental plasticity

The symbioses mentioned above are obligatory and are considered necessary for the normal development of the animal. However, symbionts can also act facultatively as environmentally-acquired factors enabling an organism to have different phenotypes depending upon the presence or the alleles of the symbiont (Dunbar et al., 2007; Tsuchida et al., 2010; Rosenberg and Zilber-Rosenberg, 2016; Tago et al., 2016).

Symbiont-mediated insecticide resistance provides an excellent example where symbionts provide an environmentally-dependent adaptive phenotype. Conventional evolutionary theories would insist that such resistance would spread slowly as an antibiotic-resistant allele is selected and then spreads through the population from parents to their offspring (see Yirka, 2012). However, symbionts provide an alternative route (Kikuchi et al., 2012; Takeshita and Kikuchi, 2017). In Japanese soybean fields, fenitrothion-degrading strains of *Burkholderia* bacteria are present at very low densities. *Burkholderia* is a natural symbiont of the stinkbug, *Riptortus pedestris*, which promotes its host's development and protects their eggs from other microbes. *Burkholderia* establish a beneficial symbiosis as the second instars ingest the bacteria from the soil (Kikuchi et al., 2011), and these symbionts remain in gut crypts for the entirety of the bug's life. Experimental applications of fenitrothion to fields have selected fenitrothion-degrading bacteria, which have gone from undetectable levels to greater than 80% of total culturable bacterial colonies. Moreover, more than 90%

of the stinkbugs grown on the enriched soil establish symbiosis with these fenitrothion-degrading *Burkholderia*. The fenitrothion-degrading *Burkholderia* strains confer to the host insects a resistance against fenitrothion that the stinkbugs otherwise would not possess. Thus, stink bug resistance to fenitrothion evolved rapidly via horizontal transmission of the symbiont from the environment (Kikuchi et al., 2007).

 Symbiotic organisms may be especially important in providing variation and plasticity to organisms such as *Acyrthosiphon pisum*, the pea aphid, a clonal species, where most eggs develop into females without any male support or genome. Here, different strains of *Hamiltonella defensa* provide environmentally mediated defense against parasitoid wasp infection (Oliver et al., 2003), and *Rickettsiella* bacteria provide protection against beetle predation by changing the color of the adult aphid from red to green (Tsuchida et al., 2010; Polin et al., 2015).

III. Developmental niche construction in the holobiont

 Niche construction emphasizes the agency of organisms to modify their environments and thereby act as codirectors of their own evolution and that of other species. The importance of this perspective to evolutionary biology was recognized by Richard Lewontin (1982, 1983), who noted that "organisms fit the world so well because they have constructed it." This idea has recently been extended by Lewontin (2000) and by John Odling-Smee (1988) and has become strengthened by theoretical population genetic and experimental findings that show niche construction to be an important factor in an organism's fitness (Laland et al., 1996; Odling-Smee et al., 1996; Laland, 1999; Odling-Smee et al., 2003; Donohue, 2005). Niche construction has been extended into ecological developmental biology as a manifestation of plasticity (Laland et al., 2008; Gilbert et al., 2015; Borges, 2017; Schwab and Moczek, 2017).

Niche construction by symbionts acquired through horizontal transmission has been studied extensively in the sympoiesis of the squid *Euprymna scolopes* with the luminescent bacterium *Vibrio fischeri* (McFall-Ngai and Ruby, 1991; Montgomery and McFall-Ngai ,1994). The adult *Euprymna* is equipped with a light organ composed of sacs filled with these bacteria. The newly hatched squid, however, has neither the light-emitting symbionts, nor the light organ to house them. Rather, the symbiotic bacteria interact with the larval squid to build its niche. The juvenile squid acquires *V. fischeri* from seawater by pumping through its mantle cavity (Nyholm et al., 2000). The bacteria bind to a ciliated epithelium in this cavity; the epithelium binds *only V. fischeri*, allowing other bacteria to pass through. The bacteria then secrete tracheal cytotoxin and lipopolysaccharide A to induce hundreds of genes in the epithelium, leading to the apoptotic death of the epithelial cells, their replacement by a nonciliated epithelium, the differentiation of the surrounding cells into storage sacs for the bacteria, and the expression of genes encoding opsins and other visual proteins in the light organ (Koropatnick et al., 2004; Chun et al., 2008; McFall-Ngai, 2008; Tong et al., 2009).

After the bacteria have induced the morphological changes in the host, the host secretes a peptide into the *Vibrio*-containing crypts which neutralizes the bacterial toxin (Troll et al., 2010). The bacteria also instruct the epithelial cells to make and secrete chitotriosidase (Kremer et al., 2013), an enzyme that degrades some of the extracellular chitin into chitobiose. Chitobiose causes the bacteria to multiply. The bacteria detach from the epithelial cells and, following the gradient of chiobiose from lowest to highest concentration, enter the pores leading to the ducts and eventually to the crypts of the nascent light organ (Mandel et al., 2012; Kremer et al., 2013). Once the *V. fischeri* are inside the crypts, bacterial products (portions of the cell wall and membrane) induce apoptosis in the squid epithelial cells that had once attracted them (Koropatnick et al. 2004). The squid crypts collect bacteria in densities high enough for them to emit light (Visick et al., 2000; Septer and Stabb, 2003). Both organisms change their gene expression patterns, and both benefit from their association: The bacteria get a niche and express

their light-generating enzymes, and the squid develops a light organ that allows it to swim at night in shallow waters without casting a shadow.

 Niche construction is also seen in the formation of the mammalian gut by the microbial populations colonizing it from the birth canal. For complete development of the mouse gut, the microbial symbionts are needed (Hooper et al., 1998). Microarray analyses of mouse intestinal cells have shown that commensal gut symbionts upregulate the transcription of numerous mouse genes to normal levels. These genes include those encoding the enzyme colipase (which is important for nutrient absorption), angiogenin-4 (which helps direct the formation of blood vessels) and Sprr2a (a small, proline-rich protein that is thought to fortify matrices that line the intestine). Thus, the "normal" amount of gene expression in the gut is that which is regulated by the microbes (Hooper et al., 2001).

 The induction of Angiogenin-4 is a fascinating story of niche construction. Stappenbeck and colleagues (2002) have demonstrated that in the absence of *Bacteroides thetaiotaomicron*, the capillaries of the small intestinal villi fail to develop their complete vascular networks. Without this bacterial species, gut capillaries--those blood vessels that are necessary for gut health and which take nutrients from the intestines and bring them to the body-- aren't properly made. Stappenbeck and colleagues also showed that *B. thetaiotaomicron* was able to induce angiogenesis in its host by inducing gene expression in the Paneth cells of the intestine. In mice without Paneth cells, the capillary network failed to form properly even after inoculation with *B. thetaiotaomicron* or conventional gut bacteria. Other experiments showed that the Paneth cells were responding to *B. thetaiotaomicron* by transcribing the gene encoding angiogenin-4, a protein known to induce blood vessel formation (Hooper et al., 2001, 2003; Crabtree et al., 2007). But angiogenin-4 has another role, in addition to its eponymous ability to form capillaries. Angiogenin-4 is bactericidal for *Listeria monocytogenes* and

Enterococcus faecalis, killing 99% of these bacteria within 2 hours. These bacteria are pathogens for mammals, and they are also competitors for *Bacteroides*. So while *Bacteroides* helps us make gut capillaries, we help *Bacteroides* by getting rid of its major competitors (Hooper et al., 2003). Molecules such as angiogenin-4 that are able to kill Gram-positive bacteria may serve to structure the bacterial community in the natural setting.

IV. Genetic assimilation and plasticity-first evolution

One of the mechanisms explaining the origin of novel and complex phenotypes has been the fixation of environmental-mediated plasticity into internal, genetic transmission (West-Eberhard, 2003; Pigliucci et al., 2006; Pfennig et al., 2010; Gilbert and Epel, 2015; Levis et al., 2018). It has even been proposed that macroevolutionary changes may have arisen through such mechanisms of genetic assimilation (Pigliucci and Murren, 2006; Jablonski, 2017). When considering microbial symbionts as agents that had once mediated phenotypic plasticity (section II), but have now become a fixed entity among all individuals in a population, we need to ask if they - and the phenotypes they generate - can be transmitted directly from generation to generation.

Returning to our developmental system of *Buchnera* and *A. pisum*, we find that the thermotolerance provided by the symbiotic allele has become a matrilinear trait passed from generation to generation. The mechanism through which this symbiont is transmitted is a fascinating variation of vertical transmission. Rather than being transmitted through the germplasm, the symbionts of the aphids are transferred by the exocytosis of the bacteria from the bacteriocytes lining the gut followed by their endocytosis by the syncytial cells of the posterior region of the blastula (Koga et al., 2012). The transfer involves three generations. The bacteriocytes releasing the bacteria to the embryos are within a developing larva that is still within its mother. Thus, within this system, the transmission of *Buchnera* appears to be strongly vertically transmitted rather than environmentally acquired.

Moran and Yun (2015) were able to demonstrate that the thermotolerant phenotype of the holobiont aphid depended upon the strain of *Buchnera* symbiont in its cytoplasm. When they replaced the heat-shock protein-deficient *Buchnera* with the heat-shock protein-producing strain, the entire aphid became thermotolerant. This replacement caused "a massive effect on host fitness" by disrupting 100 million years (about 1 billion generations) of continuous maternal transmission of the symbiont lineage. The aphid's *Buchnera* is thought to have evolved from a free-living Gram-negative ancestor similar to modern Enterobacteriacaeae such as *E. coli*. The acquisition of *Buchnera* by the aphid host allowed it to exploit the sap of vascular plants a nutrient source (Bennett and Moran 2015). Indeed, sap-feeding insects are thought to be among the first herbivores of vascular plants. The genotypes of both organisms have co-evolved such that neither *Buchnera* nor the pea aphid host can survive without one another. *Buchnera* DNA has shrunk to become one of the smallest known genomes, and it lacks genes for lipopolysaccharide production, among others (van Ham et al., 2003). It has also lost some of the negative regulatory elements controlling amino acid synthesis, allowing it to produce the essential amino acids that the host aphid cannot make by metabolism or obtain through its diet of plant sap. The genomes complement one another (Wilson et al., 2010; McDonald et al., 2011). Thus, the fitness of the holobiont came from an allele of the symbiont which is really no longer part of the environment, but a genetically assimilated feature of the organism.

While the pea aphid genetically assimilated its symbiont by making it a mitochondria-like organelle, other insects have genetically assimilated particular genes from their symbionts (Cordeaux and Gilbert, 2017). Phytophagous, leaf-eating, beetles in the superfamilies Chrysomeloidea (leaf beetles and longhorn beetles) and Curculionoidea (weevils) acquired their abilities to digest plant material through the genetic assimilation of genes encoding cellulose-digesting proteins (Kirsch et al 2014). This appears to be accomplished by three successive episodes of lateral gene transfer. Plant cell walls, rich in pectin and

cellulose, are the largest reservoir of organic carbon on earth (Gilbert, 2010). Most animals, as mentioned earlier, do not have genes encoding the enzymes that digest these plant polysaccharides (Calderon-Cortes et al., 2012). Bacteria, protists and fungi, however, evolving as plant pathogens or saprophytic detritovores, have genomes that do synthesize and secrete such enzymes. Most of these plant cell wall-digesting enzymes belong to various glycoside hydrolase families, constituting cellulases, hemicellulases, and pectinases.

 Nearly half the number of insect species feed on living plants, and they require symbiotic microbes to digest the cell walls. Genes encoding pectin depolymerases (polygalacturonidases; PGs) are not found in Hymenoptera, Lepidoptera, or Diptera. However, such genes have been found in Coleoptera (Kirsch et al., 2014). Clusters of PG-encoding genes are seen in the genomes of chrysomelid beetles and curculionid weevils. Using genomic, phylogenetic, and functional approaches, Kirsch and his colleagues sequenced and compared the DNA sequences encoding pectin depolymerases in several species of beetles. Their results indicate that the PG-encoding genes in beetles are derived by horizontal gene transfer to the host beetle DNA from three sets of symbionts. The ancestor of the phytophagous beetles appears to get its PG genes from a pezizomycotine Ascomycote fungus (such as those fungi that become lichens.) These PG genes are found in both the Curculionoidea and the Chrysomeloidea. The fungus could have originally been a symbiont, an entomopathogen, or a food source. A second set of PG genes, also derived from Ascomycota fungi, is seen only in the longhorn Lamiinae beetles; while a third set of PG genes, derived from Bacteroides bacteria, entered the genomes of the pea and bean weevils of the Bruchinae. In addition, the cellulases and hemicellulases (for the digestion of the secondary plant cell wall) of the phytophagous beetles also appear to originate from horizontal gene transfer (Pauchet et al., 2014). Kirsch and colleagues (2014) hypothesize that the horizontal gene transfers have been key events promoting the capacity of these beetles to use plants as their primary source of nutrition.

Therefore, we find that genetic assimilation from environmental microbes could have been a critical factor in the evolution of herbivorous insects, with the phytophagous beetles acquiring specific genes, while the sap-ingesting aphids assimilated entire genomes.

V. Developmental bias scaffolded by symbionts: herbivory

Developmental bias is the hypothesis that the mechanisms of development permit some variants to arise more readily than others. This can entail constraining the development of certain phenotypes and facilitating the development of others. Whereas constraints are readily detectable in normative developmental biology (Waddington, 1938; Maynard Smith et al., 1985; Galis, 1999, 2006), examples of developmentally facilitated bias are less accepted (Uller et al 2018). I would contend that some remarkable examples of facilitated adaptations can be easily seen once one views development as involving the entire holobiont, not just the zygotic genome. One obvious case becomes herbivory.

Symbionts allow the possibility of herbivory; for, as mentioned above, most animals cannot digest plant cell walls. Microbes, however, are poised to enable such herbivory because certain species are detritivores and phyotopathogens adept at such demolition and whose genomes encode the enzymes necessary to metabolize these complex polysaccharides into digestible sugars. Moreover, bacteria can also supply essential amino acids to animals whose food sources lack them, and they can detoxify the poisons that plants generate to protect themselves against such herbivory. In this way, symbionts can provide additional resources to organisms as well as removing obstacles that prevent them from transitioning to a novel (in this case, dietary) state (Itoh et al 2018). Agents that provide this type of facilitation are known as "scaffolds", a term introduced into the philosophy of biology by Jim Griesemer (2014). Here, developmental scaffolds (Chiu and Gilbert, 2015) are not just inputs (such as nutrition) or constitutive parts of developmental processes (such as regulatory networks), but

catalysts that remove the difficulties that would otherwise prevent certain developmental pathways. Such a scaffold is sensitive to and responsive to the states of the scaffolded system, such that the scaffolded system goes through its development through its interactions with the scaffold. The scaffold and the scaffolded system are distinct from one another, and in many instances, can be seen as constituting an interactive chimera. The scaffold can also, in some cases, contribute materially to the scaffolded system, even while retaining its separateness (Sterner 2017). Chiu and Gilbert (2015) have argued that microbes have scaffolded the reproduction of animals and that animals have scaffolded the reproduction of microbes, each facilitating the other.

Here, I argue that developmental symbioses have facilitated the evolutionary trajectory leading to herbivory. Once plant wall-digesting and plant toxin-neutralizing symbionts are established, the evolution of herbivory behaviors (grazing, browsing, sap-ingestion) and adaptive structures for eating plants (teeth, jaws, mandibles; microbial niches in gut) are facilitated and made more probable. The facultative developmental symbiosis that provides animals plasticity to eat plants must precede the production of herbivore behaviors and adaptive structures.

The first obstacle to this developmental bias hypothesis is the cultural bias that herbivory must precede carnivory as a mode of feeding. Vermeij and Lindberg (2000) hypothesize that "the widely held view that the overall pattern of trophic evolution in metazoans has been from plant grazers to carnivores has its origins in...such pedagogical tools as Elton's (1927) ecological pyramid and Odum's (1953) food chain." In these neontological views of ecosystems, herbivores eat the herbs, and carnivores eat the herbivores. However, genomic and paleontological evidence converge to show that herbivores are derived, and that the basal state of both Ecdysozoa and Vertebrata is carnivory (Vermeij and Lindberg, 2000). Similarly, "macrophagous primary producers" are relatively late additions to marine ecosystems, which for millions of years was dominated by filter feeders, detritovores, and the carnivores that ate them.

Among tetrapods, herbivory had to wait until the plant-digesting symbionts were established in the carnivore guts. The dental, cranial, and digestive tract modifications for plant mastication and digestion "would be relatively ineffectual in the absence of endosymbiotic microorganisms for cellulolysis." (Sues and Reisz, 1998). These plant-degrading microorganisms thrive in the leaf litter and could have been obtained by detritovores through the ingestion of plant litter (Hotton et al 1996). They could also be obtained by insectivores, through the ingestion of insects that had already been colonized by plant-eating microbes (Sues and Reisz, 1998). The transmission of such microbes might have been accomplished by juveniles consuming the feces of adult conspecifics (Troyer, 1982; Modesto, 1992). Such coprophagous transmission is also seen in contemporary termites, allowing them to digest wood (Hongoh et al., 2005). Herbivory is therefore a derived trait that evolved from carnivorous ancestors. To be an herbivore means that the animal developed a community of plant-digesting microbes in its gut.

In this "microbe-first" model of herbivory, the early herbivores lived in an "under-filled region of eco-space," (Brocklehurst, 2017), a world-wide food court. As expected, extensive adaptive radiations have been detected among the first herbivores, such as Captorhinids, who exhibited much greater morphological diversity than their sister groups that did not evolve herbivory. Moreover, the increase in the rates of morphological diversity was strongest in the areas of dentition and mandible. Bellwood (2003) and Brocklehurst (2017) contend that there is a causal relationship between the herbivorous diet and radiation.

The importance of thinking in terms of a holobiont organism is seen in the literature of plant herbivory and the "three-way interaction" (Wielkopolan and Obrepalska-Steplowska, 2016) among plants, insects, and bacteria (Hammer and Bowers, 2015). Here the plant holobiont includes the eukaryotic plant cells and their bacteria, which collectively synthesize poisons against the insects and can signal predatory wasps to kill the herbivorous invaders. Similarly, the insect holobiont includes the zoon that has the jaws to macerate plants and create

detritus for the bacteria in its gut, as well as the gut microbes that can digest plant cell walls and detoxify the poisons made by the plants.

Herbivory is widespread in mammals, and strategies of foregut, rumen, and cecal digestion have each occurred (Janis, 1976; Hofmann, 1989; Steele et al., 2016). But one of the pinnacles of herbivory in tetrapods must be the ruminant mammal, as exemplified by the cattle species, *Bos taurus*. Here, a new portion of the stomach, the rumen, has evolved to house plant-digesting symbionts. Daniels and Yohe (2015) define the rumen as "a large anaerobic fermentation chamber where plant-degrading rumen microbiota (bacteria, protozoa, archaea, and fungi) ferment otherwise non-digestible plant-based foodstuffs into primarily the volatile fatty acids acetate, propionate, and butyrate." The stomach of the cow has four main sections, and the physical growth of the rumen during calf development and its dominance over the other portions of the stomach is occasioned by fermentation products of the bacteria.

Newborn calves have sterile rumens, and the digestive tube becomes colonized by microbes as the calf pass through the birth canal. Within two days of birth, the area of the rumen is seen to have microbes within it. However, the baby calf does not receive grass or grain to eat until it is weaned. Before that time, it receives milk from the mother cow (again, one must remember that cows' milk is actually for their calves, not for humans). A calf is born with an esophageal groove, muscular folds that come together to form a tube that will bypass the rumen. The suckling reflex and milk proteins open this groove, permitting the milk to enter the abomasum portion of the stomach. When the calf starts eating grass or grain (about three weeks on American farms), the esophageal groove closes, bringing the masticated grain into the rumen (Daniels and Yohe, 2015; Baldwin and Conner, 2017). There, bacteria such as *Ruminociccus flavefaciens* produce "cellulosomes," plant wall-digesting enzyme complexes that are bound to the bacterial cell surface, which efficiently metabolize the complex polysaccharides. Over 70% of the cow's energy comes from this microbial digestion of plant fiber (Flint et al., 2008; La Reau and Suen, 2018), demonstrating the primacy of

microbes in a cow's herbivorous feeding strategy. The bacteria in the rumen multiply when given this food, and as they proliferate, they produce volatile fatty acids, including butyrate. Butyrate causes the dramatic growth of the rumen as well as the differentiation of the ruminal papillae and musculature (Sander et al., 1959; Baldwin and Connor, 2017).Thus, the gut bacteria help construct their niche, the rumen.

When one considers developmental biases in evolution, one must consider the bacteria as integral to the developing holobiont. These symbionts have facilitated the development of the structures that have enabled them to evolve into herbivores. Herbivory is a holobiont property. As Vemeij and Lindberg (2000) have noted, "The establishment of symbioses between *potential* herbivores and various microorganisms in the gut may have been a necessary *precondition* for the evolution of feeding on such plant tissues." [italics mine]. This may explain why herbivory evolved numerous times independently across the animal kingdom. There are at least five independent origins of herbivory in ants, alone, and each episode is tightly coupled with different groups of Rizobiales bacteria (Russell et al., 2009). Symbiosis appears to be a major force leading to convergent modes of morphologies and behaviors.

Whether such biases are constraints or facilitations may depend on the level or the time one observes them. Developmental constraint and facilitation in holobionts are entangled to a degree where it is difficult to separate them. Although avoiding the term "holobiont" (Moran and Sloan, 2015), Bennet and Moran (2015) have called obligate symbiosis an "evolutionary rabbit hole," a term "implying a generally irreversible journey into an odd world where the usual rules do not apply." Just as the nutritional symbiosis between the pea aphid and *Buchnera* bacteria has allowed the aphid to use an abundant and under-utilized resource--plant sap, it made the aphid dependent upon that mode of feeding. Its mandible has become so specialized that it cannot bite or chew. *Buchnera* and the aphid zoon also became interdependent in their metabolic cooperation. In silico systems analysis suggests that the metabolic network linking *Buchnera* and

the aphid are very fragile and renders the symbiont, and hence the holobiont, very sensitive to environmental changes (Thomas et al., 2009). The symbiont and the host become co-dependent, and mutations in the symbiont can cause the death of the matrilineage. Similarly, in the coral-algal symbiosis, increased water temperatures cause the death of the coral by facilitating the release of its plant symbiont (Gates et al., 1992; Fujise et al., 2014). Stable interactions create dependencies as well as new possibilities.

In 2002, Margulis and Sagan put forth a hypothesis that major trends in evolution involved "acquiring genomes." Microbial symbionts have been shown to be agents of phenotypic variation and reproductive isolation. They may also have facilitated the major evolutionary transitions to multicellularity and sexual reproduction (Alegado and King, 2014, Woznica et al., 2017). Herbivory may serve as another outstanding example whereby the acquiring of symbiont genomes facilitated the evolution of major new phenotypes of animal form and behavior.

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