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The Holobiont With Its Hologenome Is A Level Of Selection In Evolution

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Symbiosis—once thought to be a peripheral phenomenon—is the hallmark of life on Earth, from genomes through ecosystems (Gordon 2012). What concerns us here is the level of the organism. Symbiosis is replacing an essentialist conception of "individuality" with a new conception, the holobiont, which is a multilineage biological entity, and, we argue, a major level of natural selection. Zoological organisms have traditionally been defined as individuals on the bases of anatomical, embryological, physiological, immunological, genetic, or evolutionary criteria (Geddes and Mitchell 1911; Clarke 2010; Nyhart and Lidgard 2011; see Gilbert et al. 2012). These conceptions, though, are not wholly independent of one another, and each of these definitions stems from the common tenet of genomic individuality: an individual contains a single genome.

Over the past two decades, however, animals and plants have been revealed to be functional consortia of many species living, developing, and evolving together (see Zilber-Rosenberg and Rosenberg 2008; Gilbert et al. 2012; McFall-Ngai et al. 2013). This discovery that symbiosis is the rule and not the exception in animals and plants is fundamentally transforming the classical conception of an insular individuality into one in which interactive relationships among species creates a new entity, a "holobiont"—the integrated organism resulting from host cells and persistent populations of symbionts (Margulis 1993; Rosenberg et al. 2007). This notion challenges and seeks to replace the concept of a monogenomic individual whose essential identity arises during development, is maintained by the immune system, and which is selected through evolution.

The hologenome concept of evolution posits that the holobiont (host + symbionts) with its hologenome (host genome + symbiont genomes) is a level of selection in evolution (Zilber-Rosenberg and Rosenberg 2008; Rosenberg and Zilber-Rosenberg 2014). The concept is supported by a growing body of data that demonstrate (1) that the holobiont functions as a unique biological entity anatomically, metabolically, immunologically, and during development (Gilbert 2011); (2) that the hologenome is transferred with fidelity from one generation to the next; and (3) that consideration of the holobiont as a level of selection brings forth several previously underappreciated modes of variation and evolution.

Since certain specialized terms are used throughout this chapter, we would like to define them before discussing the concepts. The term holobiont was initially introduced by Margulis (1993) to describe a host and its primary symbiont. Rohwer et al. (2002) described the coral holobiont as including all of its symbiotic microorganisms, including Bacteria, Archaea, protists, and viruses. Zilber-Rosenberg and Rosenberg (2008) further generalized on the term holobiont to include all animals and plants and introduced the term hologenome to describe the sum of the genetic information of the host and its symbiotic microorganisms. The aggregate of all microorganisms of a holobiont is known as the microbiota or microbiome, a term coined by Lederberg and McCray (2001). The term "host" is used here in the classical sense to denote the larger, eukaryotic, multicellular organism in or on which the "symbionts" reside.

The Holobiont as an Anatomic Unit

The anatomical individual animal has been regarded as a structured whole. Yet, polymerase chain reaction data combined with high throughput DNA sequencing show that animals and plants "share" their bodies with numerous "species" of bacteria and other microbes. In most animals, including *Homo*, the largest numbers of symbionts are found in the digestive tract. Often, the number of symbiont cells exceeds that of the host. Although it has frequently been asserted that the number of cells in the human microbiota is ten times as numerous as the number of cells in the human body, the ratio is quite variable and closer to one (Rosner 2014). In some marine sponges, symbiotic bacteria account for around 35% of the mass of the organism (Hentschel et al. 2012). Regarding plants, bacteria are by far the most numerous colonists of plant leaves, being found in numbers up to 10^8 cells per gram, sufficiently numerous to contribute to the behavior of the individual plants on which they live (Lindow and Brandl 2003). The rhizosphere of plants contains 10^5–10^6 fungi and 10^7–10^9 bacteria per gram of soil, the highest concentration being attached to the root epidermis (Foster 1988).

When analyzing the number of bacterial species associated with a specific host, it should be noted that the estimated number of species, such as those presented in table 24.1, are minimum numbers because species representing less than 0.01% of the total population would not be detected with current methods. This reservation can have far-reaching implications since multiplication and amplification of minor species can play an important role in the adaptation of holobionts to changing conditions and also in their evolution.

The growing data bank on microbial species associated with specific animals and plants has led to certain generalizations. Host microbiota is different from the community in the surrounding environment. Host microbiota is animal or plant species specific, even in different environments (Brucker and Bordenstein 2013; Franzenburg et al. 2013). Different microbial communities dominate different tissues of the same organism. In addition

Table 24.1
Examples of bacterial species associated with animals and plants

Host	Minimum number of bacterial species
Invertebrates	
Drosophila melanogaster	209
Marine sponge	2,996
Coral	2,050
Termite gut	800
Vertebrates	
Human gut	5,700
Human skin	1,000
Bovine rumen	5,271
Great ape gut	8,914
Plants	
Phyllosphere	252
Endophytes	77
Rhizosphere	30,000

Source: Adapted from Rosenberg and Zilber-Rosenberg (2014).

to Bacteria, Archaea, protists, and viruses are also present in holobionts. We suggest the high diversity of microbes in holobionts results from the large variety of niches in different tissues and from the constantly changing environmental conditions, especially diet. In addition, bacteriophages probably prevent any specific bacterial strain from dominating according to the "kill the winner" hypothesis (Thingstad 2000).

Analyses of the microbiotas of humans indicate that there is a core microbiota, which includes bacterial species that are common to all individuals and are present most of the time in relatively large numbers (Turnbaugh, Hamady, et al. 2009). The noncore microbiota includes those species that are readily exchangeable and vary as a function of environmental condition, such as diet and disease state. It is usually the noncore microorganisms that are changeable by external manipulation (prebiotics and probiotics). Sometimes these acquired microbes can become stable inhabitants of the holobiont and part of the core microbiota (Voss et al. 2015).

The importance of microbiota as an anatomical unit has been highlighted in several organisms. What, for instance, is the entity that we call a cow? It is considered a herbivore, but without its gut symbionts—diverse communities of cellulose-digesting and fermenting bacteria, anaerobic fungi, and ciliated protists structured in its multichambered stomach—it cannot digest plant material. The symbionts have played a determinative role in its evolution (Kamra 2005).

Similarly, what we call a coral is a holobiont. In reef-building corals, the algal symbiont, *Symbiodinium*, enters into the ectoderm of their host where they transport up to 95%

of their photosynthetically produced carbon compounds to their hosts (Muscatine et al. 1984). The entry of the algae into the eukaryotic cells is facilitated by changes in gene expression (Lehnert et al. 2014). And in exchange, the coral gives the endosymbionts critical nutrients and a safe, sunlit habitat in an otherwise nutrient-poor habitat (Roth 2014). When this symbiosis is broken (for instance, by a prolonged increase in sea-surface temperatures), these corals "bleach." That is to say, they lose their algal symbionts and may die (Rosenberg et al. 2007).

Mastotermes darwiniensis, a termite of northern Australia, is especially problematic in terms of anatomical individuality. The worker termites eat the wood of trees, digesting the cellulose in their guts and constructing elaborate subterranean nests. But the worker termite cannot digest cellulose without its gut symbiont, *Mixotricha paradoxa*, which is itself an anatomical composite of at least five other species, including a eukaryotic protist, a bacterium that acts as a mitochondrion, about 250,000 *Trepinema* spirochetes that provide locomotion, a large bacillus, and about 200 larger spirochetes. Margulis and Sagan (2001,3) called it "the beast with five genomes."

The communities of microbes have specific places where they live in and on the body, their biofilms are structured, and organs have evolved to include them (Lee and Mazmanian 2010). Moreover, many animals, especially insects, contain a specialized cell type, the bacteriocyte, which often coalesces into a bacteriome, an organ for housing the symbionts. The formation of this organ can involve the recruitment of genes used for more general aspects of embryonic development (Matsuura et al. 2015).

In summary, animals can no longer be regarded as individuals by anatomical criteria. Rather, we are holobionts, integrated organisms comprised of both host cells and persistent populations of symbionts. Anatomically, individual animals must be classified in the same clades as centaurs, minotaurs, and fairies.

Integrated Physiology of Holobionts

The physiological view of animal individuality regards the organism as composed of parts that cooperate as an integrated whole (Milne-Edwards 1827; Leuckart 1851). The complexity of animal and plant organization is seen to be accompanied by the increasing division of labor among organ systems, a concept analogous to Adam Smith's conception that socioeconomic progress results from the division of labor (Limoges 1994). The present biological division of labor in animals and plants includes also their microbiota, which break down cellulose supplying energy in addition to providing amino acids, vitamins, short-chain fatty acids, and other essential materials for the holobiont (Rosenberg and Zilber-Rosenberg 2014) and much more.

Molecular research has now demonstrated that symbionts can become part of an obligatorily integrated union (Douglas 2010; MacDonald et al. 2011; Vogel and Moran 2011). For

example, the "genome" of the mealy bug *Planococcus* is the product of a nested symbiosis: animal cells harbor the Betaproteobacterium *Tremblaya princeps*, which in turn harbors a Gammaproteobacterium, *Moranella endobia*. The synthesis of amino acids is coordinated between these two microbes and the host. Three of the enzymes needed for phenylalanine biosynthesis are encoded by *Moranella*, five other enzymes are encoded by *Tremblaya*, and a final enzyme in this pathway is encoded by the genome of the insect itself (McCutcheon and von Dohlen 2011). Note, the genomes of all three organisms have been altered through this symbiosis. Such metagenomic sequencing has demonstrated the importance of microbes in other insect physiological systems (Vásquez et al. 2012; Weiss et al. 2012).

A bacterial symbiont of the pea aphid, *Hamiltonella*, provides immunity against parasitoid wasp infection (Oliver et al. 2009). But in this case, the protective variants of *Hamiltonella* result from the incorporation of a specific lysogenic bacteriophage within the bacterial genome. The aphid must be infected with *Hamiltonella*, and the *Hamiltonella* must be infected by phage APSE-3. As Oliver et al. (2009, 994) write: "In our system, the evolutionary interests of phages, bacterial symbionts, and aphids are all aligned against the parasitoid that threatens them all. The phage is implicated in conferring protection to the aphid and thus contributes to the spread and maintenance of *H. defensa* in natural *A. pisum* populations." This is not so much group selection of conspecies as team selection of consortia.

Integrated host–symbiont biochemical pathways are characteristic of mammals, as well; cometabolism has been introduced to describe the physiology of the holobiont (Smith et al. 2013). This notion reflects the findings that about one third of a mammal's metabolome has a microbial origin (Wikoff et al. 2009; McFall-Ngai et al. 2013). Microbes have even been shown to be important in synthesizing certain mammalian hormones (Yano et al. 2015). The term cometabolism was introduced (Smith et al. 2013) to describe the findings that kwashiorkor was not just a disease of protein-poor diet. Rather, the disease originated through poor diet plus certain types of bacteria. The gut bacteria take our ingested foods and convert them into new products. A person's metabolism is the result of cometabolism—a function of "microbiota and host diet" (Smith et al. 2013, 552).

The epidemic of obesity in developed and developing countries has generated a wealth of literature regarding the origin and mechanisms of this widespread phenomenon. A relatively novel connection that has been suggested is the contribution of the microbiota. It has been shown in mice, chickens, and humans that obesity is correlated with different bacterial communities. An elegant experiment by the Gordon group (Ridaura et al. 2013) demonstrated that both microbiota and diet influence obesity. Separate groups of germ-free mice, fed low-fat mouse chow, as well as diets representing different levels of saturated fat and fruit and vegetables, were infected with microbiota from obese and lean human twins. Bacteria from the feces of the obese twin caused significantly greater increase in body mass and adiposity than bacteria from the lean twin.

Bacteria may also be critical in maintaining a woman's health during the last stages of pregnancy. When bacteria from pregnant women in their third trimester were transplanted into germ-free mice, the mice became fatter and developed insulin resistance, just as pregnant women do. This did not happen with the bacteria from first-trimester pregnant women (Koren et al. 2012). Microbial symbionts appear to be a normal part of animal physiology, working toward a functional holobiont. And when birth has occurred, the woman makes food not only for her newborn, but also for the newborn's microbes. The mother's milk even contains oligosaccharides that the mammal cannot digest but which serve as food sources for the symbionts, especially *Bifidobacteria*, which has evolved a group of glycosylases specifically for digesting these carbohydrates (Sela et al. 2011; Zivkovic et al. 2011; Yoshida et al. 2012).

These examples and others demonstrate that animals and plants are not individuals by physiological criteria. Rather, they are holobionts, integrated through their metabolic networks with microbes.

Integrated Development of Holobionts

The developmental view of animal individuality (Huxley 1852) is a variant of the anatomical version of biological individuality. In this regard, the individual animal (or plant) is understood to be that which proceeds from ovum to ovum. That understanding was critically important after Robert Remak and others showed that animals were composed of myriads of smaller individuals, cells, each alive in its own right (see Nyhart and Lidgard 2011). Indeed, developmental mechanics (experimental embryology) centered on the question of developmental individuality, which E. B. Wilson (1986) considered the most important biological question of the day.

This notion of a dynamic part–whole relationship is now being extended to symbionts as part of the development of the holobiont body. Indeed, new evidence demonstrates what we understand to be a broader sense of the "individual" through the interactions of animal cells and microbes (McFall-Ngai 2002; Gilbert and Epel 2009; Fraune and Bosch 2010; Pradeu 2011). The development of both vertebrates and invertebrates is predicated from the intimate relations with microbes, and to a large degree, we "codevelop" together with our symbionts (Gilbert and Epel 2009; 2015).

In numerous organisms, the development of particular organs depends on chemical signals from symbionts (Douglas 1988; 2010). For instance, the ovaries of the parasitoid wasp, *Asobara*, require signals from their *Wolbachia* symbionts if they are not to undergo apoptosis (Pannebakker et al. 2007). *Wolbachia* bacteria are also responsible for the correct anterior-posterior patterning in the nematode *Brugia malayi* (Landmann et al. 2014). In numerous other animals, the development of certain new organs is made possible by interactions with microbes. Newborns of the squid *Euprymna scolopes* lack a light organ.

This organ is responsible for protecting the squid against predators that could recognize its shadow on the seabed (Peyer et al. 2014). The instructions for making this organ are not encoded in the genome of the squid; rather the squid embryo has evolved the ability to cooperate with one particular bacterial species, *Vibrio fischeri*. Without this bacterium, the light organ does not develop, and without the ability of the squid to concentrate *V. fischeri* in the light organ, the luminescent genes of the bacteria are not expressed (Millikan and Ruby 2001).

In vertebrates, the development of the immune and digestive systems is not completed without gut bacteria (Ley et al. 2006; 2008; Lee and Mazmanian 2010). "Germ-free" mice have insufficient intestinal capillaries, poorly developed or absent gut-associated lymphoid tissue, and a T-cell repertoire so diminished that they have an immunodeficiency syndrome (Stappenbeck et al. 2002; Duan et al. 2010). In the developing guts of mice and zebra fish, hundreds of genes are activated by the microbiota (Hooper et al. 2001; Rawls et al. 2004). These are normal induction events that are required by the developing organism. In mice, the "normal" levels of angiogenin-4 and colipase gene expression are those levels induced by the bacteria. A germ-free mouse has only 10% of the normal levels of *angiogenin-4* m RNA (encoding a protein needed for blood vessel formation), and around 2% of the normal levels of *sprr2a* mRNA, which encodes a matrix protein. In zebrafish, microbes act through the canonical Wnt pathway to regulate the normal proliferation of the intestinal stem cells (Rawls et al. 2004; Bates et al. 2006). Without the microbes, the intestine is unable to develop the normal numbers of enteroendocrine and goblet cells (Rawls et al. 2004; Bates et al. 2006), and the fish pancreas has a paucity of insulin-secreting beta-cells (Hill et al. 2016). In both fish and mice, normal differentiation and growth of the gut depends on symbiotic microbes.

What actually can be observed is a mutualistic codevelopment. The mammalian tissues signal the microbes to form regionally specific biofilms, inducing gene expression in the microbes. Furthermore, the Angiogenin-4 induced by the microbes in the mouse intestinal cells also helps the microbes. It not only makes blood vessels, it is also an antibiotic against *Listeria*, the major competitor of *Bacteroides* (Cash et al. 2006). In macaques, mother's milk allows for the growth and survival of particular bacteria that secrete arachidonic acid, an inducer of a particular subset of helper T-lymphocytes that are important in preventing infections of the newborn by *Candida* and *Salmonella* (Ardeshir et al. 2014).

One particularly interesting area of microbial effects on holobiont development involves mammalian brain formation. Germ-free mice, for example, have lower levels of NGF-1A and BDNF (respectively, a transcription factor and a paracrine factor associated with neuronal plasticity) in relevant portions of their brains than do conventionally raised mice. There are even anatomical differences in these brains. In germ-free mice, the brain microglial cells (tissue macrophages that are critical in homeostasis and disease prevention) do not complete their maturation (Erny et al. 2015). Diaz Heijtz et al. (2011, 3051) concluded that "during evolution, the colonization of gut microbiota has become integrated into the

programming of brain development, affecting motor control and anxiety-like behavior." Other investigators have noticed similar effects and have concluded that a "microbe–gut–brain" axis exists (Cryan and Dinan 2012).

The relationship between symbiotic bacteria and the development of various cognitive states is now being explored (see Bravo et al. 2011; Mulle et al. 2013). Indeed, epidemiological evidence in humans and experimental evidence in mice implicate bacteria as important in preventing the symptoms of autism (Hsiao et al. 2013). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis (Yano et al. 2015).

The microbiotas are thus integrated into the normal networks of animal development, interacting with the eukaryotic cells of their "host." Development is a matter of interspecies communication (Gilbert 2001; 2003; McFall-Ngai 2002), and animals have outsourced some of their developmental signals to their symbionts. We not only coevolve, we codevelop. From the viewpoint of developmental biology, we are holobionts.

Integrated Immunity of Holobionts

Protection against pathogens is one of the most general and important contributions of the resident microbiota to the health of animal and plant holobionts. In humans, the normal microbiota has been shown to protect against infection by pathogens in the oral cavity, the intestine, the skin, and the vaginal epithelium.

The discipline of immunology has been called "the science of self/non-self discrimination" (Klein 1982). In this view, the immune system consists of defensive "weaponry," evolved to protect the body against threats from pathogenic microbes. Accordingly, if it were not for the immune system, opportunistic infections would prevail (as they do in cases of immune deficiencies) and the organism would perish.

In a fascinating inversion of this view of life, recent studies have shown that an individual's immune system is in part created by the newly acquired microbiota. In vertebrates, the gut-associated lymphoid tissue is specified and organized by bacterial symbionts (Rhee et al. 2009; Lanning et al. 2005). When symbiotic microbes are absent in the gut, the immune system fails to function properly and its repertoire is significantly reduced (see Lee and Mazmanian 2010; Round et al. 2010). Microbial colonization is critical for the development of T-lymphocytes and B-lymphocytes in the intestinal mucosa (Olszak et al. 2012; Wesemann et al. 2013). Similarly, Hill and colleagues (2012) have shown that microbial symbionts provide developmental signals that limit the proliferation of basophil progenitor cells and thereby prevent basophil-induced allergic responses. Lee and Mazmanian (2010, 1768) conclude, "Multiple populations of intestinal immune cells require the microbiota for their development and function."

In general, most bacterial pathogens infect their animal hosts predominantly via mucosal surfaces. In addition to mechanical and immunological barriers, mucosal surfaces are protected against pathogen infection by the high concentration of microbiota colonizing

the mucosa. It has been suggested (Innerebner et al. 2011) that resident bacteria occupy binding sites needed by pathogens for adhesion and release antibacterials active against pathogens. In addition, it was shown that DNA derived from gut commensal bacteria of mice respond to foreign antigens, for example, pathogens, and activate the immune system (Hall et al. 2008).

The immune system of the holobiont therefore appears to be more a "passport control agent," or even a "bouncer," than a defensive army posted to keep the zoological organism "pure." It distinguishes, by evolutionary experience, between potential symbionts and potential pathogens (see Matzinger 1994). Indeed, the immune system actively recruits the symbionts. Peterson et al. (2007, 328) have shown that intestinal IgA, in addition to its well-known role in attacking pathogens, plays a "critical role in establishing a sustainable host-microbial relationship." Similarly, these Peyer's patch (PP) antibodies, which are essential in fighting opportunistic pathogens, appear to be involved in "the creation of an optimal symbiotic environment on the interior of the PPs" (Obata et al. 2010, 7419). Even the Toll-like receptors that mediate innate immunity are utilized by *Bacteroides* to establish a host-commensal relationship. The ability of symbiotic bacteria to use the innate and acquired immunity pathways to initiate symbioses has led Round et al. (2011, 974) to conclude that "the immune system can discriminate between pathogens and the microbiota through recognition of symbiotic bacterial molecules in a process that engenders commensal colonization." The host immune system has been co-opted to support the colonization, limitation, and persistence of *symbiotic* bacteria within the host.

Thus, the immune system, built, in part, under the supervision of microbes, does not merely guard the body against hostile organisms in the environment; it also mediates the body's participation in a community of "others" that contribute to its welfare (Tauber 2016; Dale and Moran 2006). What counts as an individual is now seen as dynamic, context dependent, and responsive to symbionts. Throughout the animal and plant kingdoms, symbionts are involved in the production of immune responses (see Gilbert and Epel 2015; Cytryn and Kolton 2011). Once in, they help keep others out.

The immune system may have evolved for the suppression of potential "cheaters," those lower level parts of the group that would proclaim their own autonomy and that would multiply at the expense of the others (Tauber 2000; 2009; Ulvestad 2007; Eberl 2010; Pradeu 2010). The problem of cheaters, then, has to be solved in such a way that associates in a symbiotic relationship are under the social control of the whole, the holobiont (Stearns 2007).

Transmission of the Hologenome between Generations

The data described above clearly demonstrate that the holobiont with its hologenome is a unique biological entity in which the symbiotic microbiota is an integral part of its fitness. For the holobiont to be considered a level of selection, both host and symbiont genomes

must be transmitted with fidelity from one generation to the next. The precise modes of vertical transmission of host genomes are well understood and need not be discussed here. However, in recent years, it has become clear that microbial symbionts can also be transmitted from parent to offspring by a variety of methods, including cytoplasmic inheritance, via eggs, coprophagy (consumption of feces), direct contact during and after birth, via insect vectors, and via the environment (Rosenberg and Zilber-Rosenberg 2011). In the numerous cases (plant and animal) of vegetative (asexual) reproduction, the microbiota is automatically transferred to offspring.

Regardless of the mechanism used, there is now good evidence that the microbial component of the holobiont is transferred with fidelity from generation to generation. Many bacterial symbionts have coevolved with their hosts for many generations. In ants (genus *Cephalotes*), for example, many members of the microbiota have been present since the diversification of the host genus in the Eocene (Sanders et al. 2014), and great apes have retained many of their microbiota by vertical transmission over similar evolutionary timescales (Ochman et al. 2010).

In humans, most of the colonization of the newborn gut occurs when the baby transits the birth channel via inoculation with maternal vaginal and fecal microbes. Furthermore, in addition to providing oligosaccharides to support particular classes of gut colonizers, human breast milk has been shown to be a continuous source of bacteria for the infant gut (Fernández et al. 2013). From the point of view of the hologenome concept, it is reassuring to realize that babies acquire microbial diversity from their mother's milk. Because some human symbionts are transmitted with great accuracy from mother to offspring for many generations, they can be used as a window onto human migration. In particular, the bacterium *Helicobacter pylori* has been used as a conserved marker of ancestry and migration (Dominguez-Bello and Blaser 2011).

Genetic Variation of Holobionts

According to the hologenome concept of evolution, genetic variation can arise from changes in either the host or the symbiotic microbiota genomes. In host genomes, variation occurs during sexual reproduction, chromosome rearrangements, epigenetic changes, and ultimately by mutation. These same processes leading to variation occur in microorganisms. Such genetic changes in the host or in the microbial symbionts can be reflected in changes of the holobiont phenotype.

In addition, genetic changes in the genome of the microbiota can occur by three further processes: microbial amplification, acquisition of novel strains from the environment, and horizontal gene transfer. These three processes can occur rapidly and are important elements in the evolution of animals and plants.

Microbial amplification involves changes in the relative numbers of the diverse types of associated microorganisms that can occur as a result of environmental factors, such as

diet, changing temperatures, and exposure to antibiotics. For example, children on a high-fiber diet have a high abundance of bacteria from the genera *Prevotella* and *Xylanibacter*, whereas children on a high-carbohydrate diet have abundant *Shigella* and *Escherichia* (De Filippo et al. 2010). Further support for amplification of certain bacteria following a change in diet comes from a study of infant gut microbiota (Koenig et al. 2010) in which changing the diet from milk to solid foods caused an alteration in infant gut microbiota with sustained increase in the abundance of *Bacteroidetes*. In a study performed on gnotobiotic mice, it was observed that a one-day change in diet from high fiber to high fat brought about an immediate change in microbiota (Turnbaugh, Ridaura, et al. 2009).

An increase in the number of a particular microbe is equivalent to variation by gene amplification. Considering the large amount of genetic information encoded in the diverse microbial population of holobionts, microbial amplification is a powerful mechanism for adapting to changing conditions. Microbial amplification at the level of the microbe is pure Darwinian selection (the result of favorable conditions), but at the level of the holobiont, amplification of a microbe is genetic variation.

Acquiring new symbionts from the environment is another mechanism for introducing variation into holobionts. Animals come in contact with billions of microorganisms during their lifetime in the food they eat, the water they drink, the air they breathe, and by direct interaction with other animals. Plants contact numerous microorganisms through their roots, the surrounding air, and also by insect vectors. It is reasonable to assume that occasionally, as a random event, one of these microorganisms will overcome the immune system, find a niche, and become established in the host. Unlike microbial amplification, acquiring new symbionts can introduce entirely new sets of genes into the holobiont.

In the pea aphid, *Acyrthosiphon pisum*, there are several species of bacteria that live within its cells. The bacterium *Rickettsiella* provides a pathway for aphid color change, turning genetically red aphids green through the synthesis of quinones (Tsuchida et al. 2010). Similarly, variants of *Buchnera* spp. bacteria provide the aphid with thermotolerance (at the expense of fecundity at normal temperatures; Dunbar et al. 2007); indeed, when Moran and Yun (2015) exchanged *Buchnera* symbionts having different thermotolerance alleles in different lines of aphids, the resulting aphid holobiont took on the characteristics of the symbiont. Thus, whether the holobiont can reproduce in hot weather or have cryptic coloration can depend not on "its" genome, but the genome of its symbionts.

Research on acquisition of microbes from the environment has focused during the past century mainly on pathogens because these harmful infections represent a key challenge to agriculture and to human health. However, many of the principles derived from studies on infection by pathogens should also apply to beneficial microorganisms. Probably, acquisition of beneficial bacteria occurs frequently but generally goes unnoticed.

Horizontal gene transfer (HGT), also known as lateral gene transfer, refers to the movement of genetic information across normal mating barriers, between more or less distantly related organisms, and thus stands in distinction to the standard vertical transmission of

genes from parent to offspring. HGT is common in bacteria but can also take place from microorganisms to animals and plants and vice versa. Examples include transfer of carotenoid biosynthetic genes from a fungus to aphids (Moran and Jarvik 2010), transfer of alpha- and beta-tubulin genes from eukaryotes to the bacterium *Prosthecobacter* (Jenkins et al. 2002), and transfer of functional cellulase genes from bacteria to a nematode (Mitreva et al. 2009). Large tracts of *Wolbachia* DNA have been horizontally transferred from these common intracellular bacterial endosymbionts to the nuclear genome of their insect hosts (Nikoh et al. 2008). In general, it is clear that introduction of genes by HGT into eukaryote genomes has been a major force propelling biological innovation and evolution.

Evolution of Holobionts

How does microbial-driven variation lead to the evolution of complexity? Microbes were the only forms of life on this planet for 2.1 billion years. During this period, they "invented" biochemistry, evolved enormous genetic diversity, and split into two domains, Bacteria and Archaea. The first eukaryote was probably formed by the uptake of bacteria to eventually form mitochondria and chloroplasts, and possibly by the uptake of an Archaea by Bacteria to form the nucleus (i.e., variation by acquisition of a microbe). Subsequent evolution of multicellular organisms proceeded both by the uptake of whole microbes and by HGT of genes from microbes into the genomes of the microbiota and into the host genome. All of the anatomical, metabolic, physiological, developmental, and immunological traits of holobionts ascribed to microbes fit into this category. Probiotics are applied examples of this principle.

An example of a major evolutionary event that was driven by the acquisition of bacteria is the ability of some animals to use cellulose and other complex polysaccharides as nutrients. Evolution of termite and cockroach hindgut microbiota may be viewed as a gradual process of internalizing microbial consortia that digest plant litter from the environment. Instead of plant debris decaying in the external environment prior to ingestion, it "rots" primarily in the hindgut after ingestion (Dietrich et al. 2014). Similar arguments have been put forth for the origin of herbivorous dinosaurs and the first plant-eating mammals.

A possible example of evolution of humans by HGT between bacteria is the ability of Japanese to digest agar because they have a bacterium in their gut that contains a gene that codes for agarose. Europeans lack this bacterium and cannot digest agar. The gene coding for agarase was obtained by HGT to a resident gut bacterium from a marine bacterium that was present on raw seaweed that is part of the traditional Japanese diet (Hehemann et al. 2010).

A key event in the evolution of placental mammals, including humans, was the acquisition by HGT from a retrovirus of the gene coding for the protein syncytin (Dupressoir et al. 2012). Originally, syncytin allowed retroviruses to fuse host cells together so they

could spread from one cell to another. Now the viral protein allows the formation of placental structures necessary for the attachment of the embryo to the uterus (Dupressoir et al. 2012). Similarly, retrovirally derived enhancers appear to have played a critical role in the generation of the progesterone-sensitive uterine decidual cell, which is also critical for maintaining pregnancy (Wagner et al. 2014). These data indicate that the integration of viral DNA into a host genome played a primary role in a major evolutionary leap, the formation of placental mammals.

Recent analysis has shown that HGT in animals and plants typically results in tens or hundreds of active foreign genes, the majority of which seem to be involved in metabolism. In humans, 145 genes (not found in other primates) were attributed to HGT (Crisp et al. 2015). These genes play a variety of roles, such as fatty acid degradation as well as antimicrobial or inflammatory responses. Most of the foreign genes identified in the study came from bacteria, but some originated from viruses and yeasts. One hundred twenty-eight genes found in land plants but absent from algae were identified as derived from prokaryotes, fungi, or viruses. Many of these genes are related to essential or plant-specific metabolic and developmental processes (Yue et al. 2013).

Role of Microbiota in Speciation

Lactobacillus plantarum is responsible for mating preference in *Drosophila* by altering the cuticular hydrocarbon pheromone concentrations (Sharon et al. 2010). It is generally accepted that mating selection represents an early step in sexual isolation and speciation (Coyne and Orr 2004). Since microbes are largely responsible for the odor of animals, it is likely they play a general role in mating preference.

Microbiota also plays a role in postzygotic reproductive success. When recently diverged wasp species were crossbred, the hybrids died during the larval stage. Antibiotics rescued hybrid survival. The authors conclude, "In this animal complex, the gut microbiome and host genome represent a co-adapted hologenome that breaks down during hybridization, promoting hybrid lethality and assisting speciation" (Brucker and Bordenstein 2013, 699). Similar results have been shown in subspecies of mice (Wang et al. 2015).

Coda

The organism as a holobiont represents a paradigm change in biology. Twenty-first century techniques have revealed that symbiotic relationships are the rule and not the exceptions. Animals and plants are holobionts consisting of the host and diverse symbiotic microorganisms. What we had thought was a monogenomic individual is actually a consortium on the anatomical, physiological, developmental, immunological, and even behavioral levels. Moreover, these microbial symbionts can be transmitted from parent to offspring by a

variety of methods, including germ-line transmission, proximate transmission (as during mammalian birth), and environmental infection. Consideration of the holobiont as a unique biological entity elicits the hologenome concept of evolution, wherein the holobiont (host + symbionts) with its hologenome (host genome + symbiont genomes) is a level of selection. Indeed, the acquisition of microbes and their genes provides powerful mechanisms for driving the origin of species and evolution of complexity. Evolution proceeds both via cooperation and competition, the two going hand in hand.

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