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ORIGINAL ARTICLE

Holobionts as Units of Selection and a Model of Their Population Dynamics and Evolution

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Abstract
Holobionts, consisting of a host and diverse microbial symbionts, function as distinct biological entities anatomically, metabolically, immunologically, and developmentally. Symbionts can be transmitted from parent to offspring by a variety of vertical and horizontal methods. Holobionts can be considered levels of selection in evolution because they are well-defined interactors, replicators/reproducers, and manifestors of adaptation. An initial mathematical model is presented to help understand how holobionts evolve. The model offered combines the processes of horizontal symbiont transfer, within-host symbiont proliferation, vertical symbiont transmission, and holobiont selection. The model offers equations for the population dynamics and evolution of holobionts whose hologenomes differ in gene copy number, not in allelic or loci identity. The model may readily be extended to include variation among holobionts in the gene identities of both symbionts and host.

Keywords Holobiont · Holobiont model · Hologenome · Level of selection · Microbiome · Microbiota · Symbiont

Introduction

This article discusses the concept of a holobiont—an animal or plant host together with all the microbes living on or in it, exosymbionts and endosymbionts, respectively. The article reviews the degree of integration between a host and its microbiota and the evidence for both horizontal and vertical transmission of microbes across host generations. It also provides a model for the evolution of a holobiont that combines microbe and host population processes.

Following Lederberg and McCray (2001), a microbiome refers to an "ecological community of commensal, symbiotic, and pathogenic microorganisms" that shares the "body space" of a host. Following Eisen (2015), a microbiome is a "microbial biome," i.e., a kind of ecosystem. In ecological terminology, an ecosystem is a community of populations together with its environment. According to the term's originators, a microbiome "not only refers to the microorganisms involved but also encompasses their theatre of activity" (Whipps et al. 1988). Subsequently, Rohwer et al. (2002) also included viruses and protists as part of the microbiome.

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Thus, we take a microbiome to be a microbial biome closely associated with a living host, and we take a microbiome plus its host to be a holobiont. Microbes that pass through the host would not be considered symbionts. Pathogens, like *Mycobacterium tuberculosis*, which live with their host for decades, would be considered symbionts, while pathogens like *Vibrio cholera*, which kill their host or depart in a few days, would not be considered symbionts. The union of all the genes in the holobiont, i.e., all the genes in the symbionts plus the genes in the host, constitutes the hologenome.

The "hologenome concept of evolution" considers the holobiont with its hologenome as a level of selection in evolution (Rosenberg et al. 2007; Zilber-Rosenberg and Rosenberg 2008). Genetic variations in holobionts can occur by changes in the host and/or in the microbiome genomes (Rosenberg and Zilber-Rosenberg 2016). The previously unappreciated contributions of microbiomes to holobiont genetic variation are the amplification of resident microbes, the acquisition of novel microbes, and horizontal gene transfer. These modes of genetic variation have been shown to play a significant role in adaptation and evolution of holobionts (Rosenberg and Zilber-Rosenberg 2016).

The hologenome concept of evolution has garnered support from many biologists (e.g., Fraune and Bosch 2010; Gilbert et al. 2012; Bordenstein and Theis 2015). It also has been criticized, the major criticism being that microbiomes may not be conserved across host generations with sufficient fidelity to play a role in holobiont evolution (Moran and Sloan 2015; Douglas and Werren 2016). A further criticism is the lack of a mathematical model to support the hologenome concept of evolution (Hester et al. 2016). Here, we address these criticisms. The treatment of these issues in this article extends and integrates our earlier contributions to a workshop on biological and social collectivities (Gilbert et al. 2018; Lloyd 2018; Roughgarden 2018a, b).

The Holobiont as a Biological Entity

A holobiont functions as a distinct biological entity anatomically, metabolically, immunologically, and during development. Furthermore, the hologenome may be transferred from one host generation to the next by various mechanisms.

The Holobiont as an Anatomical Unit

The anatomically defined individual animal has long been regarded as a structured whole. Yet, polymerase chain reaction (PCR) data combined with high throughput DNA sequencing show that animals and plants share their bodies with many species of bacteria and other microbes. In most animals, including *H. sapiens*, 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 been asserted that the number of cells in the human microbiome is ten times as many as the number of cells in the human body, the ratio is quite variable and closer to one (Rosner 2014; Sender et al. 2016).

The estimated number of bacterial species associated with a specific host should be regarded as a minimum because species representing less than 10^{-6} of the total population are not detected with current methods. The human gut, for instance, contains 10^{14} bacteria; species that are present in less than 10^{8} copies would not be detected (Rosenberg and Zilber-Rosenberg 2011). This reservation may be important because species present in low abundance may play a role both in physiological adaptation and in holobiont evolution during changing conditions (Hill et al. 2016).

The importance of the microbiome for the anatomical unit of the holobiont may be highlighted in several examples. What, for instance, is the entity that we call a cow? It is considered an herbivore, but without its rumen symbionts it cannot digest plant material. In reef-building corals, the algal symbiont, *Symbiodinium*, enters into the ectoderm of its host where it transports up to 95% of its photosynthetically produced carbon compounds to the host (Muscatine et al. 1984). And in exchange, the coral gives the endosymbionts critical nutrients and a safe, sunlit habitat in an otherwise nutrient-poor habitat (Roth 2014).

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 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, a large bacillus, *Trepinoma* spirochetes that provide locomotion, and larger spirochetes. Margulis and Sagan (2001) called it "the beast with five genomes."

The communities of microbes have specific places where they live in and on the body. For example, molecular approaches examining bacterial diversity have shown that skin microbiota is dependent on the body site, with specific bacteria being associated with moist, dry, and sebaceous microenvironments (Grice and Segre 2011), and different regions of the gut house different microbial communities (Donaldson et al. 2016). Moreover, many animals, especially insects, contain a specialized cell type, the bacteriocyte that often coalesces into a bacteriome, an organ for housing the symbionts. In at least one insect species, bacteriocyte formation involves the co-option of genes used for aspects of embryonic development (Matsuura et al. 2015).

In summary, animals, exclusive of their symbionts, can no longer be regarded as individuals by their anatomical structure. Rather, all animals that have been examined, including

ourselves, are holobionts, integrated organisms comprised of both host cells and populations of symbionts.

Integrated Physiology of Holobionts

An important general fitness contribution of the microbiome to the holobiont is performance of metabolic processes that the animal or plant cannot carry out by themselves, mainly regarding nutrient provision. Examples include nitrogen fixation in legumes (Oldroyd et al. 2011); cellulose degradation in ruminants, termites, and cockroaches (Russell et al. 2009; Watanabe and Tokuda 2010); photosynthesis by microalgae in corals, mollusks, and sponges (Rumpho et al. 2010); and oxidation of inorganic compounds in deep-sea invertebrates (Dubilier et al. 2008). In some obligatory biosynthetic processes, co-interactions between microbiotas and their host are required (Douglas 2010; MacDonald et al. 2011; McFall-Ngai et al. 2013). For example, the mealy bug *Planococcus* is the product of a nested symbiosis: animal cells harbor the bacterium *Tremblaya princeps*, which in turn harbors the bacterium *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).

Integrated host-symbiont biochemical pathways are characteristic of non-ruminant mammals, as well. A person's metabolism is a function of microbial and host enzymes. Gut microbiota play an important function in the production of vitamins and amino acids, breakdown of dietary fiber to short-chain fatty acids, and detoxification of harmful chemicals (Sekirov et al. 2010). About one-third of the total number of metabolites present within a mammal is a product of microbiota (Nicholson et al. 2012; McFall-Ngai et al. 2013). Microbes have even been shown to be responsible for affecting the production of a precursor of the mammalian hormone serotonin (Yano et al. 2015), and they are necessary for the normal proliferation of the insulin-secreting pancreatic cells of zebrafish (Hill et al. 2016).

Bacteria may be also 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 (Koren et al. 2012). This did not happen with the bacteria from first-trimester pregnant women. 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. Mother's milk contains oligosaccharides that the newborn cannot digest but which serve as food sources for its symbionts, especially *Bifidobacteria*, which has evolved a group of glycosylases specifically for digesting these carbohydrates (Yoshida et al. 2012).

These examples and many others demonstrate that symbionts interact with their hosts to determine the physiology of holobionts. The extent of these interactions and their fitness significance can vary from one holobiont species to another.

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. In fact, development of both vertebrates and invertebrates depends on the intimate relations with microbes, and to a large degree, we "co-develop" together with our symbionts. In numerous organisms, the development of particular organs depends on chemical signals from symbionts (Douglas 2010; Gilbert and Epel 2015). For instance, *Wolbachia* symbionts are critical for maintaining ovaries in the wasp *Asobara* (Pannebakker et al. 2007), and are critical for the correct polarity of early cell divisions of the nematode *Brugia malayi* (Landmann et al. 2014).

In vertebrates, the development of the immune and digestive systems is not completed without gut bacteria (Ley et al. 2008; Lee and Mazmanian 2010). In the developing guts of mice and zebrafish, hundreds of genes are activated by the microbiota (Hooper et al. 2001; Camp et al. 2014). The microbiota modulates transcription in the intestinal epithelium without remodeling the accessible chromatin landscape (Rawls et al. 2004). These are normal induction events that are required by the developing host organism. Without these microbes, the intestinal stem cells cannot divide properly, and the intestine is unable to develop the proper number of enteroendocrine and goblet cells (Rawls et al. 2004; Bates et al. 2006). In both fish and mice, normal differentiation and growth of the gut depends on symbiotic microbes.

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 (a transcription factor and a paracrine factor associated with neuronal plasticity) in portions of their brains than do conventionally raised mice. There are also anatomical differences in these brains. Diaz Heijtz et al. (2011, p. 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."

Microbiomes are thus integrated into the normal networks of animal development, interacting with the eukaryotic cells of their host. Development is, in part, a matter of interspecies communication (Gilbert 2003; Gilbert and Epel 2015),

and animals have outsourced some of their developmental signals to their symbionts.

Integrated Immunity of Holobionts

The discipline of immunology has been called "the science of self/nonself discrimination" (Klein 1982). In this view, the immune system consists of defensive "weaponry," evolved to protect the body against threats from pathogenic microbes. In a fascinating inversion of this view, recent studies have shown that an individual's immune system is created with contributions from the newly acquired microbiome. In vertebrates, the gut-associated lymphoid tissue is regulated by bacterial symbionts (Rhee et al. 2004; Lanning et al. 2005). Microbial colonization is critical for the normal development of T-lymphocytes and B-lymphocytes in the intestinal mucosa (Wesemann et al. 2013) as well as for inducing the specific lymphocyte populations that balance the immune response at mucosal surfaces (Ohnmacht et al. 2015). Lee and Mazmanian (2010, p. 1768) conclude, "Multiple populations of intestinal immune cells require the microbiota for their development and function."

The immune system of the holobiont appears to be more of a "passport control agent" or an ecological manager rather than simply a defensive army posted to keep the zoological organism "pure" (Belkaid and Hand 2014). It distinguishes between potential symbionts and potential pathogens. Indeed, the immune system actively recruits the symbionts. The mechanisms by which immune recognition of microbes promote host-microbial symbiosis remain obscure. However, several cellular recognition systems, traditionally studied as part of the host's defense against bacteria, also are used to facilitate colonization. These include the activation of pattern recognition receptors (PRRs; Chu and Mazmanian 2013) and Toll-like receptors (Round et al. 2011), and the subsequent induction of regulatory T lymphocytes (Sefik et al. 2015), and immunoglobulin A synthesis (Peterson et al. 2015). The ability of symbiotic bacteria to use the innate and acquired immunity pathways to initiate symbioses has led Round et al. (2011, p. 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."

Thus, the immune system, built, in part, under the supervision of microbes, does not merely guard the body against hostile organisms in the environment. Its primary function may be to mediate the body's participation in a community of microorganisms that contribute to its welfare, and only part of that function is the defense against potential pathogens (Tauber 2009; Eberl 2010; Pradeu 2012).

Transmission of the Microbiota Between Generations

For holobionts to be considered units of selection in evolution, both chromosomal and symbiotic microbial genes must be transferred from parent to offspring. The conserved mode of transmission of chromosomal genes is well established and need not be discussed here. Recent evidence indicates that microbial genes are also transferred from parent (primarily the mother) to offspring, although by less conserved and more diverse mechanisms (Table 1).

Although previously it was stated that there is a continuum between horizontal and vertical transmission (Zilber-Rosenberg and Rosenberg 2008; Theis et al. 2016), critical analysis has now led us to a more precise definition of vertical transmission, which allows for a division between vertical and horizontal modes of transmission. Vertical transmission should include three sub-types: (1) vegetative reproduction, (2) transmission via oocytes and seeds, and (3) "intimate neighborhood transmission" (INT). INT, such as during passage through the birth canal, should be included within vertical transmission since it occurs directly from parent to offspring without involvement of external microbes or

Table 1 Examples of modes of symbiont transmission

Mode of transmission	Examples	References
Vegetative reproduction (vertical)	Plants/some animals (coral, sponge, worm, Echinoderm)	Fell (1993), Hart (2002), Vaughn (2010)
Via oocytes (vertical)	*Drosophila*/*Wolbachia,* Aphid/*Buchneria*, sponge Plants/fungi	Baumann et al. (1995), Veneti et al. (2004), Hodgson et al. (2014), Sipkema et al. (2015)
Coprophagy (IRT & horizontal)	Many animals (termites, flies, koala, rabbits, etc.)	Osawa et al. (1993), Kovacs et al. (2006), Brune and Dietrich (2015)
Mother's milk (INT)	Mammals	Fernández et al. (2013), Jost et al. (2013), Sakwinska et al. (2016)
Physical contact starting at birth (INT & horizontal)	Most animals (mammals, amphibians, fish, reptiles, etc.)	Mackie et al. (1999), Gilbert (2014), Colombo et al. (2015), Baldo et al. (2015), Nuriel-Ohayon et al. (2016)
Horizontal	Grasses/endophytes, Squid/*Vibrio fischeri*	Nyholm et al. (2008), Tadych et al. (2014)

environment. During horizontal transmission, the microbe released by the parent mixes with a pool of other microbes of the same species in the environment, leading to the possibility of the microbe having an equal chance of infecting the host offspring of some other host parent as of infecting the host offspring from its own host parent. The acquisition of a microbiome by a host offspring probably involves both a horizontal colonization process and one or more of the vertical transmission types (Ebert 2013). The relative contribution of vertical and horizontal transfer of microbiota to offspring is unknown for most animal and plant species. What is known on the subject is summarized below.

Transmission of microbiota via oocytes and seeds is a classic case of vertical transmission. Endosymbionts, such as *Buchneria* in pea aphids and *Wolbachia* in many insects, are transferred vertically via oocytes. Vertical transmission from mother plant to offspring, via seeds, has been shown to occur in six species of herbaceous flowering plants examined (Hodgson et al. 2014), suggesting that this may be a widespread phenomenon. Fungal growth with the pollen tube is likely to be the way in which endophytes enter the developing seed (Beltran-Garcia et al. 2014). The dominant proportion of sponge-specific bacteria present in the tissues of marine sponges are maintained through vertical transfer during embryogenesis (Lee et al. 2009), although sponge-associated bacteria can also be acquired via horizontal transmission (Sipkema et al. 2015).

Vegetative (asexual) reproduction, another type of vertical transmission, takes place in many animals and plants (Fell 1993; Hart 2002; Vaughn 2010). As a consequence of vegetative reproduction, the microbiome is transferred vertically to offspring.

Although fecal samples are not a precise representation of the microbial community of the colon, they have been demonstrated to be a useful proxy of the distal colon microbiome (Gill et al. 2006; Choo et al. 2015; Stanley et al. 2015). In vegetarian or omnivore animals, eating mother`s feces (coprophagy) is practiced by many young animals, thereby obtaining the bacteria required to properly digest vegetation found in their environment (Linaje et al. 2004; Kovacs et al. 2006). Koalas use a special adaptation of coprophagy (Osawa et al. 1993); development of the young in the pouch is very slow, with the joey remaining in the pouch for five to six months and relying only on the mother's milk. When the joey is approximately five months of age, the mother produces a second type of feces (pap), which the joey eats over several days. This facilitates introduction of the appropriate gut microbiota into the developing juvenile's stomach and caecum and the subsequent digestion of the eucalyptus leaves, enabling eventual weaning from the mother. In the termite hindgut-microbiota symbiosis, feces of adult termites are fed to newly hatched juveniles by workers in the colony (proctodeal trophallaxis) (Brune and Dietrich 2015). Many insects lay eggs in their feces, which are consumed by larval offspring upon hatching (Blum et al. 2013). All these modes of transmission provide a full array of the parent's microbiota. Depending on the extent that the feces mix with microbes in the soil, the transmission is INT (e.g., koala), horizontal, or intermediate.

An example of a horizontally transferred symbiont is the squid light organ/*V. fischeri* symbiosis (Nyholm et al. 2008). Following fertilization of the eggs within the female, the embryos develop an immature light organ that is free of bacteria but has three pores leading to separate epithial-lined crypts. The female host lays clutches of hundreds of eggs, which hatch almost synchronously at dusk. Adult squid release large amounts of *V. fischeri* into the water at dawn every day. The result is that sufficient symbionts are available to colonize the hatchlings. Furthermore, the squid provides a niche in which only *V. fischeri* that emit light are able to maintain a stable association. Thus, also in this horizontal (environmental) transmission, the holobiont is reconstituted faithfully, though not necessarily exactly, by the host parent's microbiome. Another example of a reliable horizontal transmission is the horizontally transmitted endophytes in different kinds of grass, from one plant to other plants of the same species (Tadych et al. 2014).

In humans, most of the colonization of the newborn gut occurs initially via inoculation with maternal vaginal and fecal microbes when the baby transits the birth channel (INT). The first facultative anaerobic bacteria that colonize the infant gut produce anaerobic conditions in the first few days of life that allow strict anaerobes to thrive (Mueller et al. 2014). Consistent with these events are the recent findings based on metagenomics and whole-genome sequencing, combined with computational and phenotypic analyses, that at least 50 of the bacterial genera from the intestinal microbiota of a healthy individual produce resilient spores, specialized for host-to-host transmission. The spores can tolerate the change from anaerobiosis to aerobiosis and back (Browne et al. 2016). The authors suggest that spore formation is a phenotype that might facilitate vertical transmission.

Microbes are also acquired from our surroundings throughout life, including from individuals and pets with which we interact (Ying et al. 2015), contaminated water, and aerosols. For example, an intimate kiss for 10 s transfers on average 80 million bacteria (Kort et al. 2014). Dog-owning adults shared more skin microbiota with their own dogs than with other dogs (Song et al. 2013). As has been discussed previously, acquisition of microbes from the environment is a type of genetic variation in holobionts that can play a role in adaptation and evolution (Rosenberg and Zilber-Rosenberg 2016).

Breastfeeding (INT) has been shown to provide an additional route of maternal microbial transmission in humans (Jost et al. 2013), nonhuman primates (Jin et al. 2011), and

cows (Addis et al. 2016). Human milk contains ca. 10^5 bacteria per ml, composed of hundreds of species. The DNA of several bacterial strains isolated from mother's milk have been sequenced and shown to be identical to that found in the offspring (Milani et al. 2015), providing additional support for vertical transmission. Comparison of the bacterial communities detected in milk to those of the sebaceous skin found on the breast indicates that major differences exist (Hunt et al. 2011), indicating that bacterial communities in milk are not simply a result of skin contamination. PCR analyses targeting unique genes from milk-specific strains demonstrated their persistence in the infant gut for at least six months. In addition to providing bacteria, mother's milk is a continuous source of complex oligosaccharides that support the growth of the major group of these bacteria, *Bifidobacterium* species, but are not digestible by the infant (Sela et al. 2011). These beneficial bacteria contain unique genetic loci responsible for vigorous growth on these oligosaccharides (Garrido et al. 2016). The latter findings suggest a remarkable coevolution between these symbiotic bacteria and their human host, enabling gut colonization by these microbes, a colonization that benefits both.

An important question regarding the holobiont as a unit of selection is: what fraction of the genes associated with the microbiota of the mother is transferred to the offspring and for how many generations? Unfortunately, there have been few studies that compared directly the microbial genes of mother and offspring. In a rat model experiment, 70% of the operational taxonomic units (OTUs) present in dams were detected in their pups at maturity (Inoue and Ushida 2003). However, there is a problem with using OTUs to determine vertical transmission because different strains of the same species will fall within the same OTU (Moran and Sloan 2015). To overcome this problem and detect transmission of gut microbiota from mother to infant with high specificity and sensitivity, Nayfach et al. (2016) employed rare, strain-specific single-nucleotide polymorphisms (SNPs), referred to as "marker alleles," and quantified the percent of marker alleles found in a mother that were shared with her infant. A minimum of 72% of marker alleles present in mother strains were found in newborns, indicating extensive vertical transmission of gut microbiota. Furthermore, the vast majority of strains that were from the mother at four days persisted in the infants for four and twelve months. Interestingly, previously undetected strains of the same species were found at later times. It is possible that these were already present four days after birth, but at numbers too low to be detected. When the diet changed, these rare bacteria amplified. It is also possible that these bacteria were acquired by horizontal transfer from the environment.

Several studies have shown that microbiotas can be maintained for many holobiont generations. Fraune and Bosch (2007) showed that two closely related species of *Hydra* differ greatly in their bacterial microbiota. Even though these *Hydra* were kept in the same laboratory environment for > 30 years, they maintained their characteristic microbiome. The maintenance of specific microbial communities over long periods of time indicates vertical transmission and/or the epithelium actively selects and shapes its microbial community. Even if the microbes were acquired by the *Hydra* from the environment, the interaction is highly specific and reconstitutes faithfully the holobiont.

Because some human symbionts are transmitted with great accuracy from mother to offspring for many generations, they can be used as a window into human migration. In particular, the bacterium *Helicobacter pylori* has been used as a conserved marker of ancestry and migration (Dominguez-Bello and Blaser 2011). For example, the reduction of genetic diversity among humans as their distance from East Africa increases is mirrored by the genetic distances between *H. pylori* strains circulating among human populations. Such parallelism is consistent with coevolution of bacteria and their human hosts since their exodus from Africa.

Ley et al. (2008) have demonstrated that different mammals have specific and typical microbiota that have coevolved and co-diverged with them. In ants (genus *Cephalotes*), it has been suggested that many members of the microbiota have been present since the diversification of the host genus in the Eocene (Sanders et al. 2014). Over evolutionary timescales, the composition of the gut microbiota among great ape species is phylogenetically conserved and has diverged in a manner consistent with vertical inheritance (Ochman et al. 2010; Yildrim et al. 2010).

Recently, Moeller et al. (2016) used an amplicon sequencing approach that assays evolving protein-coding regions in bacterial genomes to profile strain diversity within the gut microbiomes of great apes. This fine-scale resolution allows inference of the phylogenies of closely related bacterial lineages, thereby enabling tests for cospeciation between gut bacteria and the Hominidae. The analysis revealed that clades of the common gut bacteria, Bacteroidaceae and Bifidobacteriaceae, have been maintained exclusively within host lineages across hundreds of thousands of host generations. Divergence times of these cospeciating gut bacteria are congruent with those of hominids, indicating that nuclear, mitochondrial, and gut bacterial genomes diversified in concert during hominid evolution. These data provide support for the transmission of microbiomes over long time periods, but does not exclude the possibility of multiple acquisitions.

Funkhouser and Bordenstein (2013) have argued for the universality of maternal transmission of microbiota. While maternal transmission is important and may be accomplished through a variety of means (including oocyte transmission and INT), it might be more consistent to conclude

that offspring acquire their microbiota by a variety of mechanisms, often by different types of vertical modes, but also horizontally from the environment.

Holobionts as Units of Selection: Holobionts as Interactors, Reproducers, and Manifestors of Adaptation

The holobiont is therefore an anatomically, physiologically, immunologically, and developmentally integrated entity. So, does the "holobiont" qualify as a unit of selection? Is the holobiont, as a community of organisms, an appropriate level to study evolution and evolutionary change (Lloyd 2018)? It is essential, in order to proceed in the debate about whether a holobiont is one of "the" units of selection, to review the various *distinct meanings* of the term "unit of selection." The term does not refer to a singular well-defined entity, but rather to one of four possible selective roles (and their combinations) that must be carefully distinguished, from "interactor" to "replicator" or "reproducer," to "manifestor of adaptation," and finally to "beneficiary," and that have been clarified previously (Lloyd 2001, 2017); we start this section by briefly reviewing these roles.

A distinction between replicator and interactor came originally from Dawkins (1976) and was later refined by Hull: a *replicator* is an entity of which copies are made. The concept of replicator was later refined into "reproducer" by Griesemer (2000a). "[R]eproducers are entities that have the capacity to make more reproducers, such that offspring bear relations of material overlap with their parents.… Material overlap means that reproduction involves bonds of material continuity, not merely resemblance or formal information transmission" (Griesemer 2016, p. 807). Griesemer (2005) shows that merely formal relations are problematic as stand-alone concepts of reproduction, and that at least some material overlapping parts convey or confer developmental capacities on offspring via transfer of parts—a propagule generation or "progeneration" (Griesemer 2000a), which includes retroviruses, contra Godfrey-Smith's claims (Godfrey-Smith 2009, 2011; Griesemer 2016).

In addition, the "unit of selection" may refer to an *interactor*, which is an entity that interacts, as a whole, with its environment in such a way that reproduction or replication is differential (Hull 1980). In other words, an interactor is characterized by a phenotypic trait through which it interacts, at that level, with its environment. There are downstream consequences of such interactions, reflected as fitnesses, evolutionary change, or selection; but we are not concerned with such issues at present, but rather, only with the entity doing the interacting. The claim we consider is that the holobiont itself, as an inclusive whole, is interacting with its environment through specified phenotypic trait(s). To participate "as a whole" as an interactor is to interact with the environment through a trait or traits in such a way that the alteration (in fitness, etc.) is produced in a unified fashion, i.e., where the entire holobiont reflects the interaction of the holobiont's community-level trait with its environment (at any relevant level).

There are many ways to represent the interactor, most commonly as the level or target of selection, which can occur at multiple levels of selection simultaneously in multilevel selection models (Heisler and Damuth 1987; Goodnight et al. 1992; Wade 2016), or in holobiont selection models (see frameworks in Dupre 2012; Dupre and O'Malley 2013). Thus, both the host organism and the microbiota levels can undergo selection simultaneously, with a variety of outcomes (see community genetics models such as Wade 2007, 2014).

We often think of selection as always producing adaptations, but not all interactors possess "engineering adaptations" as a result of the selection process; they may merely survive and reproduce differentially, not changing their traits. Those who view adaptation as a causally and by definition necessary outcome of a selection process adhere to a "selection-product" view of adaptation. However, another, possibly more common view, based on the evolution of newly evolved traits that "solve a problem posed" by the environment is the "engineering" notion of adaptation (Lewontin 1978; Maynard Smith 1987; West-Eberhard 1992). Accordingly, a distinct category of what "unit of selection" means has been introduced: the *manifestor of adaptation*, an entity that possesses adaptation(s) at that level, which assumes the commonly held engineering notion of adaptation (Lloyd 1992, 2017).

Finally, a notion of "unit of selection" as a *beneficiary* of the selection process comes from Williams (1966) and Dawkins (1976). On this view, a unit of selection is the entity that benefits from the process of selection, and refers only to the ultimate, *long-term* beneficiary. (This ultimate beneficiary is, to Dawkins, the "selfish gene," and this view motivates his program in evolutionary biology; but it is unclear whether this view should be universalized.)

We wish to claim that holobionts with their hologenomes can be units of selection in a variety of ways, according to this taxonomy of meanings of the term (Lloyd 2018). Much confusion has arisen in the literature as a consequence of speakers using the term "unit of selection" in different ways, so it is imperative to be clear about precisely which meaning or combination of meanings is most useful for a given purpose. As described above, the holobiont can sometimes function as a unique biological entity anatomically, metabolically, immunologically, and during development. This suggests that the holobiont can function as an interactor, since it has features or traits that bind it together as an interactive whole, in such a way that it can interact, through

its collective traits, in a natural selection process. Note that this is not primarily a claim about the holobiont as a reproducer, but only as an entity interacting with its environment through defined traits in such a way that reproduction could be differential. The hologenome may also be transferred or transmitted in material overlap from one generation to the next whether partially vertically or horizontally or some combination thereof. As a unit of selection, this additional claim suggests that the holobiont may be viewed not only as an interactor, but also as a reproducer.

Much of the philosophical discussion about holobionts and levels of selection has emphasized the replication and reproduction processes, with a focus on whether the microbial part of holobionts tends to be reproducing vertically, horizontally, or both, and if so, what that implies about holobionts as "units of selection"—really, *reproducers*. But it is also important to determine whether holobionts are acting as *interactors* or as *manifestors of adaptation* in the evolutionary process (Dupre 2012; Dupre and O'Malley 2013).

Holobionts as Interactors

Despite the fact that the holobiont lives as a community of organisms, it nevertheless stands as an "individual" by many biological standards. This community is not fixed, however, but rather is a fluctuating chimera of different species and populations, with some of the populations waxing and waning in numbers. So what ties the different species together to produce an evolutionary unit of selection as an interactor? It is the community's common functioning as an evolutionary whole interacting with its environment that characterizes it as an evolutionary interactor (Zilber-Rosenberg and Rosenberg 2008; Dupre 2012). We have in mind using contextual analysis to examine holobiont selection and evolutionary change, which was designed to be compatible with multilevel selection models (Heisler and Damuth 1987; Goodnight 2013a), and has also been used to define evolutionary individuality, i.e., evolutionary "wholes" (Goodnight 2013b). Wade has summarized the application of multilevel and community genetics (2007, 2014), in which he emphasizes the evolutionary significance of genetic interaction effects (i.e., interspecific or transpacific epistasis [i.e., not additive]) among organisms, which would differentiate coevolution from evolutionary tracking (2016). Because the evolution of mutualisms involves genetic interactions and intergenomic epistasis, wherein genes of one species interact with specific genes in another (Wade 2007), when a community acts as an evolutionary whole, this can involve the deepest levels of genotypic by genotypic by environmental (G × G × E) interactions across species.

The point we are making is that, contrary to the idea that is fundamental to the one genome/one organism concept, the biological entities that can form reproducing and evolving lineages are not necessarily the same as the entities that function as wholes in their environments. As Dupre (2012, p. 160) states, the entities that function as wholes in wider biological contexts are meant as interactors. "It will immediately strike evolutionists that this conceptually separates the organism (functional whole) from the evolving entity (part of a lineage).... What I have been calling organisms are units of selection, objects between which natural selection selects."

The notion that the community of organisms of a holobiont acts as a functioning whole is essential to the idea that it could be an interactor. One example is that aphid, bacteria, and phage combine as a functioning whole to repel a parasitoid that threatens each of them—aphid, bacteria, and virus (Oliver et al. 2009). This community acts as an integrated consortium undergoing selection. Cases like this can be explored using models from population and community genetics, as Drown et al. (2013) did, treating the community represented by the holobiont as the group, with its own traits and heredity (Wilson 1980; Goodnight 1990a, b, 2005; Okasha 2006; Sterelny 2011; Drown and Wade 2014). Dupre and O'Malley's use of "collaborators" or "polygenomic consortia" has the advantage of encompassing both competition and cooperation (2013, p. 314; see also Wade 2014 for population genetic models limiting inter-genomic conflict).

A community-level trait distinguishes the holobiont evolutionary interactor and its interaction as a whole with its environment—whether biotic or abiotic, or both. "The microbial symbionts represent diverse genomes; and those genomes can also be co-selected together with the genome of their host," especially if they are faithfully transmitted, whether vertically or partially horizontally (Gilbert et al. 2012, p. 330; Zilber-Rosenberg and Rosenberg 2008; Sterelny 2011). And genetic models tell us that in large populations, complete vertical transmission of the symbionts will evolve from horizontal transmission as long as there is epistasis for host-symbiont fitness. From an initial starting point of vertical transmission at very low frequency (approximately 1% vertical and 99% horizontal), a gene favoring increased vertical transmission in either host or microbial symbiont genome will hitchhike to fixation when there are mutants with positive trans-specific epistasis in either genome (Drown et al. 2013). Since mutualisms are based on reciprocal beneficial fitness effects, there will be evolutionary pressure to evolve vertical transmission; as Drown et al. (2013, p. 52) put it, "vertical transmission is an evolutionary attractor."

Both philosophers and biologists often invoke a requirement that transmission be purely or almost purely vertical (Godfrey-Smith 2009, 2011; Moran and Sloan 2016; Doolittle and Booth 2017). But that is like drawing a line between asocial and social species at the point of evolution of sterile castes—just as there are lots of precursors in sociality before

the evolution of sterile castes, there are similarly holobionts prior to what might be called the "eu-holobiont" which has purely vertical transmission. The sterile caste and the "eu-holobiont" are only the most extreme cases on their own relative continua; complete vertical and complete horizontal transmission are the endpoints on the holobiont continuum. For example, we have lichens, most of which require two components, a fungus and an alga or cyanobacterium. The fungus and photosynthetic partner typically co-disperse, and when a piece breaks off the colony, all three components co-disperse together. Thus, the two components are typically vertically reproduced, while all three components reproduce by breaking off, all together, vertically. The lichen is in between a loose community of organisms and a "eu-holobiont," but would still be on the holobiont "continuum" (Sanders et al. 2014).

Booth objected to the "imprecision" of application of the interactor concept to the holobiont, by Dupre (e.g., 2010, 2012), arguing that details about "just what kind of causal interactions among parts serve to bind independently reproducing populations into interactors" was lacking (Booth 2014, p. 670). Just as in group selection where we have a variety of mechanisms that align groups towards being interactors, such as cannibalism and reciprocal altruism (Wade 2016), we also have at the holobiont collaborator level a collection of mechanisms that align the status of the holobiont, including niche construction and scaffolding (Laland et al. 2008; Caporael et al. 2014); structural connections; metabolic interactions such as the supplying of microbiota to digest cellulose; functional integration; collaboration; fitness-affecting interactions; and developmental scaffolding, wherein elements needed for holobiont development are provided by its environment (Wade 2007; Dupre and O'Malley 2013; Caporeal et al. 2014; Griesemer 2014a, b, 2015; Chiu and Gilbert 2015), but Booth seems to reject these mechanisms as not sufficiently precise for his purposes.

It is clear that Booth favors the views of Godfrey-Smith, who defines his "Darwinian population" as a combination of an interactor and a reproducer (Godfrey-Smith 2009, 2011). Admittedly, the holobiont is a more complex entity to hold the "interactor" label than any preceding it. But interactors are not necessarily identical entities as reproducers; keeping these roles separate and distinct is one of the virtues of the analyses of Hull (1980), Griesemer (2000b), and Lloyd (2017). The chief reason this is important is that holobionts and their members replicate in a variety of different fashions, and these different ways lead to different evolutionary dynamics, which can therefore not be read directly off of the interactor dynamics alone.

For example, in aphids, symbiotic bacteria provide selectable allelic variation, such as thermotolerance, color, and parasitoid resistance that enable some hosts to persist better under different environmental conditions (Dunbar et al. 2007; Tsuchida et al. 2010; Gilbert et al. 2012). Moran and Yun (2015) demonstrated that alleles of symbiotic *Buchnera* bacteria confer differential thermotolerance to the pea aphid holobiont and that the replacement of one allelic population of these bacteria with another has a massive effect on host fitness. Similarly, resistance to certain insecticides is provided to the stinkbug *Riptortus pedestris* by its ingestion and incorporation of fenitrothione-degrading strains of *Burkholderia* bacteria during its larval stages (Kikuchi et al. 2012; Tago et al. 2015) Allelic variation has been found to exist within human gut microbes and this confers the differential ability of the human holobiont to digest certain complex polysaccharides (Hehemann et al. 2010, 2012). Variation in the interactor traits is selected in different environments, and importantly, reliably inherited across generations through both vertical and horizontal transmission, in various combinations. Again, this is all kosher according to the genetics of transmission of symbionts, provided that the degree of vertical transmission does not drop too low, to where the system becomes unstable, i.e., where the net breakdown by horizontal transmission exceeds the buildup of correlations by epistatic selection acting on the host and symbiont, the exact percentage depending on the degree of epistasis between the genomes; the actual process involves a continuum of co-transmissibility (Drown et al. 2013; Wade and Drown 2016). It is also important to point out that the genetic influences on the co-transmission can owe to genes either in the host genome or the symbiont genome (or both), as Drown et al. (2013) point out.

Another significant role of holobionts in evolutionary dynamics comes in the context of mechanisms of speciation. Identical hosts can have dissimilar microbiotas, thus producing reproductive isolation between populations of holobionts. Selective mating preferences, long believed to play a central role in the emergence of new species (Coyne and Orr 2004), have been shown to depend in some cases on the microbiota. In the case of diet-induced mating preferences in *Drosophila*, the symbionts from different nutrients seem to regulate pheromones necessary for mating preferences (Sharon et al. 2010). Thus, the holobiont is the interactor, i.e., the entity under selection, and not the fly or the microbiota alone.

Reproductive isolation through symbionts can also result from cytoplasmic incompatibility. When recently diverged wasp species were interbred, their offspring were nonviable at the larval stage (Brucker and Bordenstein 2013). If treated with antibiotics, however, the offspring were viable, thus demonstrating the crucial role of the microbiota in producing the incompatibility of the two species (Brucker and Bordenstein 2012). The original co-adapted hologenomes and holobionts broke down during the hybridization, thereby showing the importance of the fully adapted holobiont to speciation.

Thus, holobionts certainly can be seen as a unit of selection by the criteria of being "interactors" in evolutionary processes.

Holobionts as Reproducers/Replicators

The notion of a "replicator" or "reproducer" is an alternative category to that of an "interactor" in defining what constitutes a "unit of selection," and much of the philosophical argument concerning holobionts has centered around how well the holobiont fulfils this role. Zilber-Rosenberg and Rosenberg claimed in 2008 (p. 731) that the "hologenome is transmitted from one generation to the next with reasonable accuracy," thus advocating the concept of the holobiont as a reliable reproducer. Griesemer's "reproducer" approach admits a wide range of developmental systems in which not all hereditary resources need be located in genes, cells, or even organisms, and may extend to their nearby "environments." Griesemer (2016) views most or all life cycles as scaffolded, i.e., supported by a variety of developmental resources, where scaffolding is inherently collective, "even if the collected entities are not necessarily (all) cells or even living. What differentiates kinds of reproducers on our account is modes and mechanisms of scaffolding rather than the fact (or not) of it" (see Caporael et al. 2014). There is a clear context for niche construction here, as well, as the holobionts construct an environment in which reproduction of various kinds is scaffolded and differentiated (Odling-Smee et al. 2003; Fussmann et al. 2007; Chiu and Gilbert 2015). In other words, the various supports during development created through scaffolding often involve elements of the organism's environment, resulting in niche construction. Some holobionts may reproduce better in certain self-constructed niches than others, thus favoring some elements while disfavoring competitors in a non-random fashion (Gilbert et al. 2010; Laland et al. 2014).

While the usual objections to the existence of widespread mutualism in the biological world rest on the threat of "cheaters," i.e., organisms or genes that, in their self-interest, foil the adaptive process of a jointly adaptive state of a higher-order mutualism, we disagree that this is an accurate theory of the natural world. Despite the expectation, on this theory, that few mutualisms would be found, and more than a few cheaters (see Sachs et al. 2014; Weiblen and Trieber 2015), actual well-defined cheaters have yet to be found in nature (Jones et al. 2015; see; Bronstein 2015). Moreover, cases of parasitism transitioning to mutualism are not possible under genomic conflict theory but are common in some clades (Bronstein 2015). But rather than challenge the basic theoretical presuppositions that produced those predictions, they remain unchallenged, and an assumption is made that the cheaters do exist in nature, as yet undiscovered, while the abundant mutualisms that are found are the exceptions. One of two assumptions is made. The first is that cheaters are really present in nature but are as yet undiscovered. The second is that adaptations to suppress cheaters are more effective than standard theory predicts; the cheaters should exist, but some other adaptation (like policing, or selective rewarding) is keeping them from appearing. This second ad hoc theory (Sachs et al. 2004) adds further implausibility to an already empirically endangered approach.

In contrast to this picture, Wade and colleagues, utilizing the results of thousands of multilevel selection procedures, both in the wild and in the laboratory, and hundreds of multilevel and community genetics models (detailed in Drown and Wade 2014; Wade 2016; Wade and Drown 2016), have arrived at a rather different explanation under an ordinary set of assumptions. Drown and Wade (2014) explore the differences between biotic and abiotic environments on the evolutionary dynamics of a genetic system; we get a "runaway coadaptive process," within biotic environments like those encountered in holobionts, under typical constraints. Most of the dynamical space is taken by systems that culminate in mutualistic processes; there is only a small number of states in which the so-called "cheater" dynamics are in evidence (Drown and Wade 2014). This is very important for understanding the ubiquity of holobionts in the natural world, which are explained by these dynamics. Thus, there can always be developmental or ecological support involved in forming and sustaining a holobiont. Note that this includes retroviruses.

Our analysis together with that of Griesemer (2016) contrasts with that of Godfrey-Smith (2009, p. 39), which does not include such entities, because they do not reproduce or replicate in the correct manner. Godfrey-Smith's view rests on his definition of "Darwinian populations," which he defined as ''a collection of causally connected individual things in which there is variation in character, which leads to differences in reproductive output and which is inherited to some extent.'' How well a particular population fulfils these requirements of Darwinian individuality can be a matter of degree. But we see clearly here that Godfrey-Smith conflates reproduction (the reproducer) and selection (the interactor, the entity which possesses "variation in character," and which "leads to differences in reproductive output") into his single concept of a "Darwinian Population." This encourages one to look for the means and methods of reproduction when focusing on interactors, a surefire way to become confused when examining holobionts, because of the complexities of their microbial reproduction, and the different and multiple levels at which selection occurs.

Godfrey-Smith also limits holobionts to vertical reproduction, which apparently violates the suggested community genetics requirements for such communities, promoted here, which instead allow a range of transmission modes to represent the mutualism similar to that we find in holobionts

(Brandvain et al. 2007; Drown et al. 2013; Drown and Wade 2014; Wade and Drown 2016). Drown et al. write:

> We found that a high rate of mutation in the genes responsible for the host-symbiont fitness interaction is important to the fixation of vertical transmission. There is evidence of high rates of substitution in intracellular bacteria that are vertically transmitted, consistent with this prediction (Douglas 2010). Faster rates of evolutionary change at loci responsible for the interspecific interaction should also be observed as elevated levels of sequence divergence between symbiont populations or species. (Drown et al. 2013, pp. 54–55)

Note that the Doolittle and Booth "song not the singer" approach (2017), in which the focus is largely on interactions between holobiont and environment, rather than on the roles and entities involved in these interactions, is consistent with this aspect of the models.

As Drown et al. also note, "The transition from horizontal to vertical transmission mode may be associated with a switch from pathogenic to mutualistic interactions" (2013, p. 55). This is because increased vertical transmission would more closely align the interests of the host and symbionts (Brandvain et al. 2007; Brandvain and Wade 2009), and potentially reduce virulence (Ewald 1987; Bull et al. 1991). In addition, in theory, the co-inheritance of host and symbiont genomes via vertical transmission increases the efficiency of selection, through decreasing the amount of genomic conflict (Wade and Goodnight 2006; Wade 2007). But with purely vertical transmission, "the organism has no opportunity to escape from the consequences of any deleterious impacts it might have on its partner" (Douglas 2010, p. 68). Still, limited amounts of horizontal transmission can eliminate selection favoring reduced virulence, by breaking down host-symbiont relations. Drown et al. (2013) propose that,

> The statistical character of gene combinations determines whether host and symbiont fitnesses covary positively or negatively. This in turn determines whether or not selection favours "inter-specific gene combinations" (positive fitness covariance) or an antagonistic, evolutionary arms race between host and symbiont (negative fitness covariance). (2013, p. 55)

Wade (2014) explored when this would be the case in maternally passed-on microbiomes.

Thus, holobionts can be reproducers, both horizontally and vertically and in combinations of those modes. It is also possible that microbial amplification (Zilber-Rosenberg and Rosenberg 2008) is a key source of holobiont variation, as it involves changes in the relative or absolute numbers of diverse types of microorganisms in the microbiota of a given holobiont, as has been modeled by Osmanovic et al. (2017; Soen 2014). They show through a population genetics-like model of holobionts that selection of toxin-resistant bacteria over one generation of hosts leads to stress-dependent increase in the tolerance of the host's offspring. Adaptation of the holobiont itself through community selection of bacterial communities occurs with further exposure to the toxic stress (Soen 2014; Osmanovic et al. 2017).

When the frequency of a microbe within a holobiont changes, this is the parallel to variation by genic selection, as the additional microbes are essentially adding more alleles to the holobiont. When we look at the holobiont as a unified whole, and consider its entire microbiota, we can see changes in the frequencies of its microbial populations as a mechanism potentially for adapting to new environmental conditions, as shown in Soen (2014; Osmanovic et al. 2017). At the level of individual microbes, ordinary individual-level interactor selection occurs, but at the holobiont level, amplification of a microbe can amount to an increase in (holo) genomic variation, a feature of the interactor, as well as of the reproducer, over its lifecycle (Wade 2014). As we will see in our model later on, there are distinctions among gene copy-number selection, allelic selection, and locus selection.

Acquiring genetically diverse strains of microbes from the environment into the host is another way to introduce variation into holobionts, in the evolutionary process. (But we don't know if the colonization of the host by diverse strains of microbes from the environment influences the evolutionary process unless after colonization they become somehow heritable.) These may be random events, but when successful, can introduce entirely new symbionts and their genes into the holobiont through its microbiota (see Kikuch et al. 2012).

A third method of rapid holobiont diversification is through horizontal gene transfer (HGT) in which alleles are transferred between genomes of different entities, sometimes even entities from different kingdoms. While HGT is commonly seen among bacterial strains, it is also seen moving hereditary material from microbiota to plants or animals. One well-studied example is *Wolbachia*, a bacterial endosymbiont, whose DNA has been found inside the nuclei of insects, and where, in at least one species, it has importantly given rise to a sex chromosome (Nikoh et al. 2008; Leclercq et al. 2016).

All three sorts of holobiont diversification resulting from the introduction of variation into the microbiome can be reproduced/replicated to future generations. Such continuation of the variation is necessary to keeping the genomic variation introduced by the microbiota relevant to the evolution of the holobiont.

Part of the controversy concerning whether holobionts can be "units of selection" rests on a conflation of the question of whether an entity is an "interactor" with whether it is a "reproducer." Once these categories are kept separate,

holobionts emerge often and can be modeled as both well-defined interactors and well-defined reproducers in evolutionary processes. This leaves us with a final question.

Holobionts as Manifestors of Adaptation

Can holobionts be manifestors of adaptation? Can they exhibit enough unity of structure to exhibit a trait as something that might have been selected as an engineering adaptation benefiting the collected holobiont as a whole? Again, a manifestor of adaptation requires the addition of a change in trait design at some level in some organism in the holobiont due to a course of natural selection. In first considering the holobiont, Zilber-Rosenberg and Rosenberg (2008) thought that the holobiont had "its own specific properties that are not necessarily the sum of those of the host plus its microbiota." Consider the example of placental mammals.

Acquisition by HGT from a retrovirus of a crucial gene coding for the protein syncytin was a key event in the evolution of placental mammals (Dupressoir et al. 2012). What syncytin does is allow the proper formation of the placenta. Similarly, the origin of the progesterone-responsive uterine decidual cell that characterizes eutherian mammals was also generated by retroviral DNA (Wagner et al. 2014). Thus, the integration of viral DNA into a host genome played a primary role in a major evolutionary leap, the formation of placental mammals. The viruses, as parts of the microbiota of the host, the ancestral predecessor of the mammals, in *interaction* with those ancestors, resulted in something entirely new: the placental mammal. We would find it hard to think of a more impressive engineering adaptation than placental fusion to attribute to a holobiont and its microbiota. Placental mammals are thus "manifestors of adaptation," where the relevant holobionts in the evolutionary story are their predecessors, the pre-placental mammalian ancestors with their retroviral DNA, and the current placental mammals.

The widespread infection of insects with *Wolbachia* symbionts provides further cases of HGT that may be considered manifestors of adaptation under these definitions (see Dobson et al. 2002; Weeks et al. 2007; Hedges et al. 2008; Teixeira et al. 2008; Nikoh et al. 2014). They can be mutualists or parasites, and in many species defend the host against viruses (Fenn and Blaxter 2006).

However, not all interactors are manifestors of adaptation, because adaptation does not follow automatically from a selection process, but requires a pattern of selection over time to produce an engineering adaptation, an accumulated change in a trait to solve a specific environmental challenge. But there may be a variety of cases of holobionts as manifestors of adaptations; the question requires further study. Caution is required: it was precisely the confusion and conflation of the question of whether something was an interactor with whether it was also a manifestor of adaptation that led to a great deal of trouble in both the group and species selection debates (Lloyd 2017).

Model of Holobiont Population Dynamics and Evolution: An Initial Sketch

A mathematical model might help to clarify conceptually how the many component processes affecting holobiont evolution jointly function. We now sketch a mathematical model for how holobionts can be said to evolve.

Existing literature presenting theory relevant to holobiont evolution is not extensive. Theory for mutualistic networks involving plants, animal pollinators, and animal seed dispersers might apply to the relations among microbial strains and their host (Bascompte and Jordano 2006; Bastolla et al. 2009). Theory for community genetics, and for the extension of the classic idea of heritability to encompass an ecological community, may be relevant to the evolution of the microbiome (Bailey et al. 2006; Shuster et al. 2006; Whitham et al. 2006; van Opstal and Bordenstein 2015; see also, Goodnight 1990a, b).

Several studies have applied classical Lotka-Volterra species-interaction models to the ecology of the microbiome. Regressing *changes* in abundance against abundance has long been used as a way to measure species interaction coefficients among microbes (e.g., Gause [1934]1964; Vandermeer 1969; Drake 1991; Fukami and Nakajima 2011; cf. Roughgarden 1998, pp. 109–120, 227–232, 345–354). More recently, Mounier et al. (2008) measured the Lotka-Volterra interaction coefficients for a microbial community in cheese using a regression technique. Stein et al. (2013) further developed the regression methods for estimating Lotka-Volterra species-interaction coefficients and used their methods to analyze DNA sequencing and metagenomic data for mice intestinal microbiomes. In the same vein, Fisher and Mehta (2014) estimated Lotka-Volterra interaction coefficients for the microbiomes in the guts of two persons, and presented graphs of the interaction webs of 14 microbial species. These webs showed an assortment of positive and negative species links. The authors identified certain species as keystone species based on the number of interaction links leading into and/or out of them, and suggested that differences between the gut microbiomes of different people could be explained by their having different keystone species. Coyte et al. (2015) suggested that competitive rather than cooperative interactions predominate among microbes in order to realize ecological stability.

In a different vein, a recent study presenting an agent-based computer simulation of community assembly for interactively equivalent species reports that an inverse relation exists between microbial biodiversity and the amount of the

parental versus environmental components in the microbiome (Zeng et al. 2015, cf. Hubbell 2001).

The problem of holobiont evolution also bears similarity to the discussion of cyto-nuclear coevolution between mitochondrial and chloroplast genes and the nuclear genes of the cells they reside in (Birky et al. 1983; Birky 2001; Rand et al. 2004; Ballard and Rand 2005; Meiklejohn et al. 2007; Smith 2007). Moreover, the importance of horizontal gene transfer among bacterial has led to discussion and models that view genes as a shared genomic resource, or public good (Galtier 2007; McInerney et al. 2011; Polz et al. 2013; Fullmer et al. 2015).

Finally of note, the relations between the gut microbes and the host tissue have been explored theoretically by Schluter and Foster (2012) and Macke et al. (2017). Moreover, Sofonea et al. (2015) have developed a general model for multiple infections to explain how the interaction among multiple parasites affects their virulence, recovery, and transmission rates with respect to host epidemiology.

Our approach complements the existing studies by offering a mathematical model derived from a population-biology perspective that focuses on the hologenome and on holobiont evolution rather than microbiome community structure, cyto-nuclear relations, or host/gut-microbe epidemiology.

The microbiome consists of at least two types of strains classified with respect to their mode of transmission. The "resident microbes" consist of strains that reside within a holobiont and its descendants and that are transmitted vertically by various mechanisms. These microbes may also participate in a horizontal transfer among their holobiont hosts forming metapopulations. The defining feature of the resident microbes is that they do not exist as a pool outside the host and are found outside their hosts only temporarily while migrating among the hosts.

The "transient microbiota" consist of strains that belong to free-living species pools in the environment. Each generation, these microbes colonize newly produced holobionts and may exchange microbes with their species pools during the life of their holobiont hosts. These microbes are not transmitted vertically from a holobiont directly to its descendants. The descendants of any given holobiont might, however, wind up having a microbiome resembling that of their parent, but only as a result of both the parent and descendants being independently colonized from the same species pools. The transient microbial strains view their host as a "habitat" (Knowlton and Rohwer 2003; Costello et al. 2012; Moran and Sloan 2015; Zeng et al. 2015; Douglas and Werren 2016).

Accordingly, the hologenome can be partitioned into three components: the host genome, the genomes of the resident microbiota, and the genomes of the transient microbiota. Different processes underlie evolutionary change in each of these components.

To be sure, strains may exist that might be called "semi-resident"—strains that combine features both of the resident component in being transmitted vertically *and* of the transient component that is in continuous horizontal communication with a free-living species pool in the environment. It seems best to begin the modeling with the resident/transient dichotomy and to extend the formulation to cover semi-resident strains later if necessary.

For conceptual brevity, we envision that the host genome consists of a set number of loci. The alleles at any given locus may change over time in the host population in ways described by classical population genetics, a process we refer to here as "allelic selection."

Turning to the resident microbiota, we envision that each microbial strain also has a set number of loci. As with the host genome, the alleles at any given locus in any particular microbial strain may change over time through classic allelic selection. Furthermore, the abundance of any microbial strain within the holobiont may also change. Hence, the resident component of the hologenome can change over time for two reasons: the alleles at the various microbial loci can change, and also the numbers of microbes carrying any particular gene locus can change. We call the change in gene copy number resulting from changes in microbe abundance "copy-number selection."

Zilber-Rosenberg and Rosenberg (2008) have termed the change in gene copy number within a holobiont "microbial amplification." To avoid confusion with the gene amplification and gene duplication processes caused by special DNA replication mechanisms, this section adopts the terminology of copy-number selection instead of microbial amplification.

Finally, microbes in the transient microbiota can vary in the alleles at the loci in each strain, in the number of copies of each locus depending on the abundance of each strain, and in addition, can vary in locus identity depending on what strains of microbes colonize the holobiont from the environmental source pools. The arrival of a new microbial strain can introduce new kinds of loci into the holobiont. Conversely, the departure of a microbial strain, or its substitution by another, can delete certain loci from the hologenome. Changes in the kinds of genetic loci in the holobiont due to the arrival and departure of different microbial strains in the transient component of the microbiome is here termed "locus selection."

Taken together, we can classify the processes that affect the evolution of the hologenome as allelic selection for the genes contributed by the host, allelic selection plus copy-number selection for the genes contributed by the resident microbiota, and allelic selection plus copy-number selection plus locus selection for the genes contributed by the transient microbiota.

Holobiont evolution and ecology are closely intertwined. Allelic selection is described by classic population genetics.

Copy-number selection adds within-host microbial population and community dynamics. Locus selection then adds species colonization and extinction dynamics. And all these within-holobiont processes take place under the umbrella, so to speak, of selection at the level of the holobiont as a whole. Thus, modeling holobiont evolution is uniquely challenging. The modeling is challenging because elements from ecological and evolutionary theory must be integrated in ways that have no ready precedent in the literature. The classic separation between ecology as the science explaining organismal abundance versus evolution as the science explaining organismal properties is blurred for holobionts.

Although a holobiont may be thought of as an individual in some sense, it is clearly a complicated individual. A holobiont is clearly an interactor, to use terminology from the preceding section, because variation among holobionts is subject to selection at the holobiont level. Whether, or in what sense, a holobiont is also a reproducer is more problematic. Whether variation in the hologenome is vertically inherited depends on the fraction of the hologenome's variation that derives from the resident versus transient component of its microbiome, and on the ratio of cell numbers carrying the microbiota's genome relative to the cell numbers carrying the host genome. That is to say, unless most of its microbiome is resident, then a holobiont is not a unit of selection, in the sense of a replicator. In the simple conceptualization in this article, a holobiont whose microbiome consists entirely or mostly of transient microbes is like an individual whose traits are acquired rather than inherited and will not qualify as a unit of selection at the holobiont level.

Model—Qualitative Specification

We now offer perhaps the simplest possible model for holobiont evolution. The model sketched in Fig. 1 is specifically for a holobiont with a resident microbiome consisting of

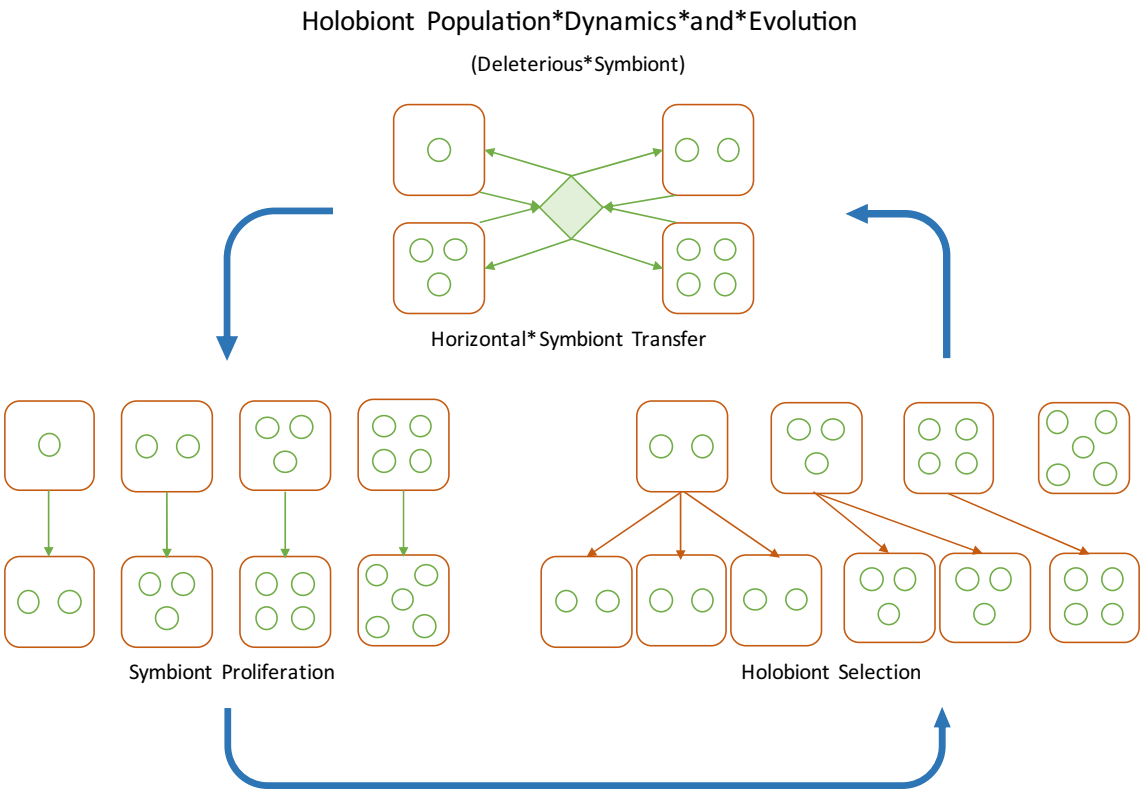

Fig. 1 A model for holobiont population dynamics and evolution where the resident microbiome consists of one microbial strain. The macro time step begins with stage 1 in the center of the figure at the top, with the horizontal exchange of microbes among hosts. The diamond in the center represents that pool of migrant microbes. Moving down and to the left for stage 2, the microbes reproduce within the host for a set number of micro time steps. Moving to the right for stage 3, the holobionts reproduce with vertical transmission of their microbiota. The number of progeny a holobiont produces depends on the number of microbes within it. The holobiont progeny become the initial condition for the next macro time step. The macro time step is iterated to generate a prediction of how the holobiont population size and the microbiome's composition change through time. This example depicts the case where the microbe is deleterious—holobionts with few microbes produce more progeny than holobionts with many microbes. If the microbe were beneficial instead of deleterious, the third stage of the figure would be reversed to show that holobionts with many microbes produce more progeny than holobionts with few microbes. The microbiomes among all the hosts comprise a metapopulation whose subpopulations within each host are bound together through horizontal transfer

one microbial strain, and where the selection at the holobiont level consists solely of copy-number selection without allelic selection or locus selection. Future work will add allelic selection for the host and resident microbiota. Also, future work will offer a model for the transient component of the microbiome, leading eventually to a synthetic model that pertains to both components of the microbiome simultaneously and includes all three modes of selection, allelic, copy-number, and locus selection.

Three processes occur in the model of holobiont evolution considered here: microbes can migrate from one host to another, microbes can proliferate within their host, and the holobionts can show differential survival and/or fecundity depending on the abundance of microbes within the host.

Although all three processes may occur simultaneously, a useful conceptual simplification is to suppose that they occur sequentially. Start with horizontal between-host migration of the microbes. Follow with within-host population dynamics of the microbes. Conclude with holobiont selection featuring differential survival and/or fertility of entire holobionts depending on the composition of the microbiome with them and assuming vertical transmission of the microbiome.

A sequence of these three processes can take place during each generation of the host. The host's generation time provides a macro time step. Within each macro time step, micro time steps occur reflecting the kinetics of microbial exchange among hosts and the generation time of the microbes. If the host is, say, an invertebrate that reproduces annually, and if the microbes divide weekly, then the macro time step would be a year and the micro time step would be a week. Fifty-two micro time steps would occur during the microbiota proliferation stage before the model could proceed to the holobiont selection stage.

The model for selection at the level of the holobiont differs from a classic model for individual selection at the level of the host because the fitness of an individual holobiont (denoted later as $W(n)$) depends on its hologenome, i.e., it depends not only on the host's nuclear genes, but also on the genes of all its symbionts.

Here are the detailed assumptions for each stage within a macro time step:

Stage 1: Horizontal Microbiont Transfer Stage

Each microbe has a given probability of exiting its holobiont and entering a "transfer pool." Then the total transfer pool is divided equally across the holobionts. Each holobiont receives the same number of transfers. Holobionts that had few microbes to begin with receive a net increase in microbe number; holobionts with many microbes to begin with incur a net decrease in the number of microbes. Horizontal transfer tends to homogenize the microbiomes among the holobionts. Horizontal transfer of microbes implies that a microbial strain is a metapopulation—a population of subpopulations, each of which resides in a host.

Stage 2: Microbiont Proliferation Stage

The population of microbes within each holobiont grows according to an ecological population-dynamic model with density dependence. The density dependence indicates that the microbiome ecologically responds to its host as a finite resource. A typical population-dynamic model for a single strain of microbes is the logistic equation used in population biology. If the microbiota consists of more than one strain, then various models of species interactions from community ecology, such as Lotka-Volterra equations, may be used instead at this stage. The population-dynamic model at this stage is iterated for as many micro time steps as needed to fit into the macro time scale.

Stage 3: Holobiont Selection Stage

Each holobiont reproduces as a whole. The number of progeny a holobiont produces depends on the number of microbes in it. The microbes are transmitted vertically from the parent holobiont to the daughter holobionts such that the number of microbes in each daughter holobiont equals the number of microbes present in its parent. Holobiont reproduction and survival is independent of the density of holobionts. The assumption of host density independence avoids introducing extraneous ecological claims about the interaction of different holobiont species with one another. This model is about the dynamics and evolution of a single holobiont species, not a community of interacting holobiont species, a topic left for future research.

These three stages are repeated for each macro time step. Iteration of the macro time step generates a prediction of how the holobiont population size and the hologenome frequencies change through time.

The online-only appendix (Online Resource 1) presents the mathematical formulation of the model, a numerical illustration of the model's predictions, a table of the parameter values used in the numerical illustration, a series of figures presenting results of the numerical illustration, and a computer program written in Mathematica to iterate the model's equations. (The computer program is available in the online supplementary material.)

Model—Discussion of Results

The three runs presented in the appendix illustrate how the processes of horizontal microbe transfer, within-host microbe proliferation, and holobiont selection combine.

Within each macro time step, the microbes proliferate within their host as their abundance approaches the carrying

capacity of the holobionts for microbes. Then, holobiont selection reduces the fraction of holobionts that contain many microbes because, in this example, the microbes are assumed to be deleterious. In this case, selection against holobionts with many microbes counters microbe proliferation within holobionts, leading to a stationary distribution of microbiome composition across all the holobionts.

The particular stationary distribution attained depends on the initial condition. Horizontal microbe transfer tends to homogenize the microbiota among the holobionts, leading each holobiont to approach the population average of microbes per holobiont. But that population average is arbitrary and depends only on an initial condition. In the model, horizontal transfer is selectively neutral by itself. That is, horizontal transfer is not biased toward or away from holobionts that already contain a large number of microbes. As long as the horizontal transfer is selectively neutral, the composition of the microbiome reflects the vagaries of history that determine the initial condition in addition to the deterministic biological processes of microbe proliferation and holobiont selection.

However, if the hosts all have the same carrying capacity for microbes, and if the number of micro time steps within a macro time step is very large, then an abundance of microbes in every holobiont approaches the holobiont's carrying capacity for microbes. In this special case, the horizontal transfer does not alter the distribution of holobiont composition because all the holobionts have the same composition to begin with.

Of course, if no horizontal transfer of microbes occurs among the hosts (which in the model is obtained by setting the migration probability to zero), then the model's picture of holobiont evolution reduces simply to clone selection among the hosts. In this special case, the winning host is simply the type with the lowest microbe carrying capacity when the microbes are deleterious, and is the type with the highest microbe carrying capacity when the microbes are beneficial. In general though, horizontal transfer binds the collection of microbiomes into a unified system, a metacommunity, rather than a collection of independent communities.

The microbiomes of the human intestine and other sites in the human body have been described as possessing ecological stability and resilience to perturbation (Lozupone et al. 2012; Relman 2012). Relying on a Lotka-Volterra species interaction model for microbiomes, Coyte et al. (2015) argue that microbiome ecological stability arises from interaction webs in which competitive relations predominate. However, the interaction webs presented by Fisher and Mehta (2014) show an assortment of both positive and negative species links. In contrast, this study suggests that horizontal transfer can confer ecological stability to microbiomes. As every traveler knows, gut bacteria readily migrate among individuals through the water supply and from the environment generally. This horizontal transfer homogenizes the microbiomes across people and can potentially dampen any tendency for the microbiome in a particular person to oscillate or exhibit other types of dynamic instability.

The model here offers equations for the population dynamics and evolution of holobionts with hologenomes that differ in gene copy number, not in allelic identity. An extension to the model may readily include variation in the allelic identities in both the microbe and host genomes. Such an extension might address questions such as the conditions for the coevolution of cooperation between the microbiota and host. The coevolution of integrated biochemical function based on gene products from both host and guest genomes might be investigated theoretically in this way.

The model diagrammed in Fig. 1 might also be modified by omitting the vertical transmission stage and adding instead a pool of free-living microbes. These microbes would have to colonize their hosts anew whenever the host population reproduces. Such a model would apply to the transient component of the microbiota, and could supplement the model presented here for the resident component of the microbiota.

A model extended to include both the resident and transient components of the microbiota could explore the relative fitness of a colonizing microbe versus a vertically transmitting microbe. Vertical transmission by a microbe is an evolved trait. For example, the zooxanthellae in some coral species have evolved to be contained within their host's gametes (e.g., *Porites cylindrica* and *Montipora digitata*; Hirose and Hidaka 2006). These zooxanthellae then form the resident component of the coral's algal microbiome. However, the zooxanthellae in a majority of reef-building corals have not evolved transmission through their hosts' gametes and instead remain as part of the transient component of the coral's microbiome (Babcock et al. 1986; Trench 1993). The extended model could show when it is advantageous for microbes such as zooxanthellae to belong to the transient versus the resident component of the microbiome. The host, of course, must cooperate in the vertical transmission process, and so the origin of holobionts that contain a resident component to their microbiome must be viewed as a result of coevolution between the microbes and the host. The evolution of vertical transmission for members of the microbiome would seem to be a critical precondition for the subsequent evolution of the extensive cooperative physiological and developmental integration of the microbiome with their hosts as detailed in the first section of this article. Moreover, the coevolution of vertical transmission of the microbiome with the host is what subsequently allows the holobiont to be considered as a unit of selection itself, because with a resident component to its microbiome the holobiont qualifies as both an interactor and a reproducer.

Many coevolutionary questions involving the microbial strains with one another and with the host may be addressed with extensions of existing coevolutionary theory in ecology (e.g., Roughgarden 1983; Brown and Vincent 1987; Dieckmann and Law 1996; Rezende et al. 2007; Carmona et al. 2015). However, vertical transmission of the resident component of the microbiota introduces a consideration largely unaddressed by existing coevolutionary theory. Indeed, while the multilevel selection approach may be similar to kin selection on standard models (Goodnight 2013a), they are not identical. The latter is an equilibrium approach, whereas multilevel selection theory is designed to identify the rate of change in traits resulting from selection acting simultaneously at multiple levels (for the mathematics, see Lloyd et al. 2008).

This article documents that the holobiont is a unit of evolutionary selection. Holobionts constitute functional wholes that are well-defined interactors, and are often replicators/reproducers and manifestors of adaptation as well. The mathematical model presented here helps to understand how holobionts evolve, looking at the processes of horizontal symbiont transfer, within-host symbiont proliferation, vertical symbiont transmission, and holobiont selection.

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