

Holes in the Hologenome: Why Host-Microbe Symbioses Are Not Holobionts

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ABSTRACT The advent of relatively inexpensive tools for characterizing microbial communities has led to an explosion of research exploring the diversity, ecology, and evolution of microbe-host systems. Some now question whether existing conceptual frameworks are adequate to explain microbe-host systems. One popular paradigm is the “holobiont-hologenome,” which argues that a host and its microbiome evolve as a single cooperative unit of selection (i.e., a superorganism). We argue that the hologenome is based on overly restrictive assumptions which render it an approach of little research utility. A host plus its microbiome is more effectively viewed as an ecological community of organisms that encompasses a broad range of interactions (parasitic to mutualistic), patterns of transmission (horizontal to vertical), and levels of fidelity among partners. The hologenome requires high partner fidelity if it is to evolve as a unit. However, even when this is achieved by particular host-microbe pairs, it is unlikely to hold for the entire host microbiome, and therefore the community is unlikely to evolve as a hologenome. Both mutualistic and antagonistic (fitness conflict) evolution can occur among constituent members of the community, not just adaptations at the “hologenome” level, and there is abundant empirical evidence for such divergence of selective interests among members of host-microbiome communities. We believe that the concepts and methods of ecology, genetics, and evolutionary biology will continue to provide a well-grounded intellectual framework for researching host-microbiome communities, without recourse to the limiting assumption that selection acts predominantly at the holobiont level.

Without doubt, the discipline of microbiology is being transformed by advances in sequencing technologies. We are now able to determine the taxonomy and functional capabilities of individual microorganisms and complex microbial communities independent of cultivation, relegating the vexations of “the great plate anomaly” (1) to history. The consequences of this technological revolution are rippling beyond the field of microbiology, most notably to the study of animals and plants. For the first time, we now have unequivocal evidence that healthy animals and plants are routinely colonized by large numbers of microorganisms, often of considerable diversity. For example, an atlas of the microbial communities associated with different regions of the human body is now available (2), and multiple other lines of evidence show that the compositions of these microbial communities vary with human genotype, health status, diet, and age (3–6). There is growing interest in microbial interventions to improve human health, as well as for applications in veterinary science, aquaculture, and crop production (7–9).

These scientific discoveries, made possible by sequencing technologies, have led some to question whether the conceptual framework and methodologies of ecology, genetics, and evolution are adequate as a research approach for understanding microbe-host interactions. It has been argued that there is a need to “upgrade fundamental theories” because host microbiome systems “raise the discussion of individuality and organismality beyond its historical perspective to a level that challenges and extends current thinking” (10). A view gaining in popularity for this upgrade is the concept of the holobiont and hologenome (11–13). In a nutshell, it is argued that the “holobiont” (host plus its microbiome) and its constituent “hologenome” (the totality of genomes in the holobiont) are a unit of selection, and therefore this unit has properties similar to an individual organism, i.e., it is a superorganism. In some cases, this concept has been applied without full consideration of its relevance or assumptions to topics ranging from the

causes of honey bee decline (14) to developmental biology (15) and cancer (16).

In contrast to the hologenome perspective, we argue here that the fields of ecology, genetics, and evolution are a perfectly adequate, and indeed more effective, conceptual framework for investigating the ecology and evolution of host-microbiome systems. In particular, a perspective that considers the host-microbiome as an ecological community (17–20) is, in our opinion, a more productive approach to investigate host-microbiome systems. The hologenome approach focuses on one level of selection (the holobiont as a “superorganism”), and as a result it is concerned with cooperative and integrative features of the “hologenome” to the exclusion of other kinds of interactions. A more complete understanding of host-microbiome systems will be gained by approaches that consider all members of the community as evolutionary entities that experience different selection pressures, potentially live in a different range of habitats, and vary in their fidelity to the association. Community ecology principles, such as successional theory, multitrophic interactions, and disturbance ecology, can be applied to host-microbiome systems (17), and there are a wide range of studies that have considered host-microbe systems in an ecological and evolutionary context, in-

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cluding the application of community ecology approaches to human microbiomes (17–25).

To evaluate the relevance of the hologenome concept to host-microbe association research, we address two key issues in this review: the central importance of modes of transmission and partner fidelity, including the use of phylosymbiosis as a proxy for partner fidelity, and the role of different levels of selection in host-microbe associations. We then consider recent application of the hologenome concept to host speciation through symbiosis, and we conclude with a brief consideration of terminological issues, including some alternative terms that do not presuppose that the host-microbiome association is evolving as a unit. Another recent critique of the hologenome concept is found in reference 26. A critique of the application of the hologenome approach to host speciation (27) is found in reference 28, along with a response by the authors, R. M. Brucker and S. R. Bordenstein, in reference 29.

MODES OF TRANSMISSION AND PARTNER FIDELITY

Partner fidelity refers to the stable association of host and symbiont genotypes across multiple host generations. Partner fidelity can be generated by vertical transmission of microorganisms or, for horizontally transmitted microorganisms, by strong genotype-dependent partner choice. Partner fidelity is a prerequisite for the hologenome, because the host and its microbial partner(s) can only evolve as a unit if they cooccur across multiple host generations, with tight host genotype-to-microbe genotype matching.

In this section, we consider the evidence for partner fidelity, first in horizontally transmitted associations and then in vertically transmitted systems. Many cases of symbiosis involve acquisition of microbes from the environment each generation, and these can involve substantial free-living populations as well as the host habitat. Examples of horizontal transmission include the rhizobia in legume root nodules, luminescent *Vibrio fischeri* in the bobtail squid *Euprymna scolopes* and the associated water column (30), soil-acquired *Burkholderia* in stink bugs (31), and the human microbiome (20). Partner fidelity is weak for many horizontally acquired symbioses. For example, the gut microbiota in genetically defined strains of laboratory mice and *Drosophila melanogaster* varies among laboratories, and also within one laboratory, over time (32–35); studies on the gut microbiota in human twins have revealed either no effect of host genotype or effects that are restricted to specific members of the gut microbiota (5, 36).

In principle, partner fidelity can be favored in systems with horizontal transmission where the hosts provide their offspring with symbionts and where specificity is high (i.e., a very restricted taxonomic diversity of microorganisms can colonize the host). Some associations appear to conform to this expectation. For example, females of some stink bug insects release a fecal pellet bearing bacterial symbionts adjacent to deposited eggs and the larval hatchlings feed on these pellets, resulting in mother-to-offspring transfer of gut symbionts (37); larvae of the hydrothermal vent tubeworm *Riftia pachyptilia* are colonized by specific bacterial symbionts which penetrate through the skin to colonize the internal symbiont organ (38); and the bobtail squid *Euprymna scolopes* discriminates against most *Vibrio* bacteria during establishment of symbiosis (39). Congruent phylogenies of the host and microbial partners can be indicative of partner fidelity but should be interpreted with caution. First, some of these analyses use slowly evolving sequences (e.g., the bacterial 16S rRNA gene) and therefore are insufficiently sensitive to detect the matching of specific

host and microbial genotypes that would be necessary to demonstrate tight partner fidelity on ecological time scales. Second, the actual patterns of transmission have not yet been well-characterized in many systems, as illustrated by recent discoveries of vertical transmission in systems previously thought to entail exclusively horizontal transmission (40, 41).

The weak partner fidelity of many symbioses can be considered to restrict the scope of the hologenome: if the collective set of genomes varies within and between host generations, then it cannot be a coherent unit of selection. Fidelity of association among genetic variants of hosts and microbes is therefore crucial in determining to what extent they may evolve as a unit. This concern has been countered by advocates of the hologenome concept with the notion of phylosymbiosis (27), a term coined to describe congruent relationships between host phylogeny and community diversity clustering metrics (e.g., Unifrac distances). The latter is an ecological measure for comparing composition (type and abundance) of microbial communities and is not a phylogenetic analysis of individual members of the microbiome. It is argued that a similar clustering pattern of microbial diversity and host phylogeny is indicative of coadaptation (10). The approach has problems at several levels. First, a reasonable null hypothesis for the community distance and host phylogenetic distance relationship is likely to be concordant clustering. Related host species share similar physiologies, microbial defense mechanisms, and biochemistries, and therefore they would be expected to have similar communities. Second, a community structure distance concordant with its host phylogenetic distance is not indicative of coadaptation. As mentioned above, it can simply reflect a common environment. A necessary (but not sufficient) condition for coadaptation to occur is phylogenetic concordance of host and microbial genotypes, which is not established by these coarsely scaled community metrics that are not the same as phylogenetic analysis. At least as practiced, phylosymbiosis using 16S bacterial sequences is not conducted at a fine-enough resolution to determine whether specific microbial genotypes show fidelity of association with particular hosts. For example, two bacteria with identical 16S ribosomal sequences can differ considerably in their genetic repertoire and differ dramatically in their ecophysiology (42). Fidelity at the level of genotypes is much more relevant for determining whether a host and microbe can evolve mutualistic coadaptations. Therefore, showing phylosymbiosis between a host and its microbiome at the level of an ecological distance metric is simply not sensitive enough. Such diversity metrics using 16S sequences more likely reflect broad associations of particular microbial groups with particular (host) environments that are conducive to growth of those microbes, rather than being indicative of an evolving “holobiont.” Phylosymbiosis does not establish that genotypic variants of microbial associates are transmitted vertically or are maintained by mutual host-microbiome partner choice, conditions necessary to establish a host genotype-microbial genotype association that promotes them to coevolve effectively as a cooperative unit. The observation that particular microbial 16S types are typically found in association with particular host species, or (even more weakly) that the community diversity metrics differ in a predictable way between host species, does not establish coinherence. And even when coinherence occurs for a subset of the microbial associates, it is unlikely to be so for all members of the community, and so it seems difficult to imagine that the entire microbiome should be

considered part of a “hologenome” with its host if only a subset of microbes meet the requisite conditions.

Phylogenetic correspondence of hosts with gut microbial communities has been demonstrated for three species of *Nasonia* jewel wasps (27), five species of great apes and humans (43), and between epithelium-associated bacteria in seven species of *Hydra* (44). However, the examples are based on 16S sequences and rely on similarity in diversity estimates among related host species, rather than strict phylogenetic concordance of individual microbial taxa with that of the hosts (which requires finer-scale genotyping). Other methodological issues include the observation that results can depend on the sequencing depth and β -diversity metrics used in the analysis (28) and that some instances of phylogenetic concordance may be driven by diet (45).

The expectation of tight partner fidelity may be met by symbioses with obligate vertical transmission, including bacterium-derived organelles (mitochondria and plastids) and some insect-microbe symbioses. For example, the phylogenies of most aphids and their maternally inherited gammaproteobacterium *Buchnera aphidicola* are perfectly congruent over an evolutionary history of ca. 100 Ma (46). However, even in systems with inherited microbes, differences in transmission of the nuclear genome (biparental) and cytoplasmic genome (maternal) can result in fitness conflict (47), which is important to their evolution but is largely neglected in the hologenome approach.

LEVELS OF SELECTION

Selection can act at multiple levels of biological organization. Selection at higher organizational levels is predicted to be significant where there is a strong overlap of selective interest among the constituent entities, e.g., genes on a chromosome, cells in the body of a multicellular organism, honey bees in a hive (48, 49).

For a host-microbiome association to be the unit of selection, the hologenome concept requires (near-)perfect concordance of selective interests both among the microbial partners and between the microbiota and host. As conflicts of interests among partners increase (e.g., due to weak partner fidelity), then the host-microbiome is undermined as a single unit of selection. Here, we consider, in turn, the selective interests of microbial partners with three different lifestyles: horizontally transmitted microorganisms with either a persistent or fleeting free-living phase, and vertically transmitted microorganisms. As described above, many horizontally transmitted microorganisms are acquired *de novo* from the environment at each host generation, often from substantial free-living populations. The ecological success of such microorganisms is linked to their phenotypic flexibility and capacity to exploit different habitats, but key aspects of the global biology of these microorganisms often are uncertain. In particular, the contributions of the different habitats (host and external environment) to the total fitness of the microbial partner are generally unknown but may vary among microbial taxa and with ecological circumstance, e.g., the abundance and distribution of suitable hosts, or the abiotic conditions and biotic interactions in the free-living environment. The most appropriate framework for investigating the fitness of these microorganisms is the entire set of habitats they occupy, not just the host-microbe association. This important aspect to biology of many microbial associates is neglected in the hologenome approach.

The host is likely to be of greater significance to the fitness of microorganisms that have transient, sometimes fleeting, external

populations (e.g., the many obligately anaerobic bacteria in the mammalian gut) than prolonged, proliferative external populations (e.g., rhizobial symbionts of leguminous plants). However, the absence of extensive free-living populations does not necessarily create a strong overlap in selective interest between horizontally transmitted symbionts and their host. Although the long-term persistence of horizontally transmitted microorganisms is linked to host availability, the selective interests of many microbial partners are not completely concordant with host fitness. For example, symbionts can promote host health and longevity, but not host reproductive fitness (45, 50–52). Male-killing symbionts and cytoplasmic incompatibility bacteria (53) are examples of other widespread symbionts that are maternally inherited but reduce host fitness. The treatment in the hologenome concept of the host-microbe association as a single unit of selection fails to address the important differences in the selective interests of hosts and their horizontally transmitted symbionts. These differences in selection between the host and its microbial partners in horizontally transmitted associations are compounded by the often-divergent selective pressures of different microorganisms within a single host. For example, antagonistic interactions can occur among different mutualists within the same host (19, 21, 22). There is, thus, conflict at two levels: between the host and its microbial symbionts and among different symbionts, and either or both of these types of conflict can undermine the alignment of selective interests among partners. The hologenome concept is, as a consequence, an unsuitable framework for considering the great majority of horizontally transmitted associations.

Given the conflicts that can arise with horizontally transmitted symbionts, we are left with associations with obligate vertical transmission as candidate hologenomes. Partner fidelity is imposed by vertical transmission, and the microbial partners have a strong selective interest in the reproductive fitness of the host because their fitness is tied to the reproductive success of the host. The selective interests of host and symbiont are not, however, in perfect alignment, because transmission of the symbiont is usually maternal, resulting in fitness conflicts between the nuclear and maternally inherited genomes over sex determination (47). Many vertically transmitted microorganisms (e.g., *Wolbachia*) distort the sex ratio to favor female offspring (53). Similarly, mitochondria variants in many plants induce cytoplasmic male sterility, which can lead to suppressive nuclear genotypes to restore pollen function (54). This coevolutionary “arms race” between nuclear and mitochondrial genomes is not predicted or expected by holobiont theory.

A second arena for conflict in vertically transmitted systems is among the microbial cells, for example, in competition for host resources, including nutrients and space, and access to the next host generation. This conflict diminishes with declining genetic diversity of the microbial populations. Where studied, the genetic diversity of a vertically transmitted microbial taxon within any host individual is minimal, largely because a small number of microbial cells are transmitted to each offspring. However, this solution to the problem of among-microbe conflict creates a different problem: a very small effective population size and resultant genomic deterioration of the microbial partner (55). Where the host benefits from (or is dependent on) its microbial symbiont, the accumulation of deleterious mutations, pseudogenes, and ultimately gene loss in the microbial partner occurs by drift, a process known as Muller’s ratchet (56). It is countered only by strong

selection acting on the symbiont for particular functions and by selection for microbial function operating at the level of the entire association, i.e., the hologenome. However, selection at this higher level is not particularly effective at preventing the massive genomic decay of various bacteria to genome sizes of <1 Mb, with some symbiont genomes of 0.1 to 0.2 Mb, comparable in size to bacteria-derived organelles (57), including in one bacterial symbiont, *Hodgkinia*, the evolutionary diversification of lineages with complementary genetic capabilities (58, 59). In some instances, host compensation for genomic decay of required microbial symbionts has led to shared metabolic pathways, with the shuttling of metabolites between host and symbiont partners (60–63).

In summary, current understanding identifies the dominant processes shaping the evolutionary trajectory of vertically transmitted microorganisms to operate at levels of selection lower than the association. We do not rule out the less parsimonious but interesting hypothesis that selection at the species or clade levels may favor lineages that solidify mutualistic dependencies with a symbiont. However, established evolutionary methods are fully able to address multilevel selection (48, 49), without the constraints imposed by the specific assumptions of the hologenome concept. Furthermore, such higher levels of selection are most likely to apply only to particular host-symbiont pairs with high partner fidelity and not to the entire host-microbiome, which includes diverse interactions and fidelities of association.

We further note that the evolution of biochemical (or other) dependencies among host-microbiome communities does not require obligatory vertical transmission and associated Muller's ratchet processes of genome decay. The loss of particular genes that produce required products in a microbe or host can occur when other members of the host-microbiome provide the product. This can result in dependency and codependency within an assemblage. But this process is not particularly mysterious and does not require the system to be evolving as a hologenome. Such biochemical dependencies are found throughout nature (64). They follow from a simple paradigm of “use it or lose it”; when a nutrient is routinely provided by the (biotic or abiotic) environment, mutational degradation or selection for biochemical efficiency can lead to loss of genes in the particular biochemical pathway. Recent modeling suggests this process can readily occur with “leaky products” produced by different members of a microbial community when they are routinely associated with each other (64). Such processes can lead to interdependent communities (65) without requiring selection at the community (holobiont) level.

MICROBIOMES AND HOST SPECIATION

Various researchers have argued that symbiotic microorganisms play an important role in speciation of animals and plants (66, 67), and it has been argued by some that such effects support the hologenome concept (27). Potential examples of microbial influences on host speciation include *Wolbachia* spp., which induce reproductive isolation between species (68, 69), or the evolution of parthenogenetic species (70), but a more general role of symbiosis in speciation had previously lacked any compelling example (67). In an exciting recent discovery, Brucker and Bordenstein (27) established that hybrid lethality between two species of insects (jewel wasps of the genus *Nasonia*) depends crucially on the presence of their microbial associates, which show unregulated growth in hybrids. Germ-free hybrids do not suffer the high levels of lethality observed in standard crosses. This finding could have

much broader implications to the causes of hybrid incompatibility and speciation, a fundamental area of research that has largely ignored the potential role of host microbiomes. However, Brucker and Bordenstein (27) further argued that the microbiome of each species represents a coevolved hologenome which is disrupted in hybrids because mismatching bacteria and host genomes cause breakdown of the system. Alternative interpretations that do not necessarily require the system to be a holobiont (i.e., cooperative unit of selection) include the following: (i) hybrids are unhealthy and therefore vulnerable to bacterial overgrowth (28), (ii) there are general disruptions in the immune system due to hybrid incompatibilities which allow bacterial overgrowth, and (iii) specific host genes (e.g., for antimicrobials) affect the growth of particular bacteria, and segregation of these matching genotypes in the hybrids results in overgrowth of particular microorganisms. The hypothesis which requires the most complex set of assumptions is that the system is a highly coevolved hologenome in which the microbiome has evolved to maximize fitness of the holobiont; this cooperative unit is disrupted in hybrids, as proposed by Brucker and Bordenstein. Hypothesis iii involves specific host-microbe gene interactions but does not require coadaptation of microbe and host. For example, the wasps could be immunologically adapted to control the growth of environmentally acquired microbes that are themselves not coevolved with the wasps. And if coevolution has occurred, it need not be cooperative but could be the result of an antagonistic microbe-wasp coevolution.

An important question for any host-microbiome is how the different microbial associates are acquired. *Nasonia* spp. are small parasitoid wasps that lay their eggs in the pupa of various flies, and the wasp species differ in their preferences for different fly species (71). If the microbiome changes dramatically when wasps develop in different fly species, it would undermine the concept of a coevolved hologenome. Even if a restricted subset of microorganisms show true coinheritance with the host, others may not, and therefore again the total system cannot be considered a “hologenome.”

This point illustrates a general problem of the hologenome approach. Even when particular host-microbe partners meet the stringent conditions for evolving as a unit of selection, the diverse array of microbes within a host encompasses a much broader range of relationships and, therefore, the entire community is unlikely to evolve as a unit of selection. The hologenome is fragile, and the concept is unlikely to have generality. Simply put, entire microbiomes do not evolve as single units with their hosts.

TERMINOLOGY

One appeal of the “hologenome” is that it provides a nifty term for referencing the host plus microbiome. This may account for its recent use in some host-microbiome papers (14–16, 72–74), even when these microbial communities did not meet the narrow conditions necessary for a hologenome. The terms “holobiont” and “hologenome” elevate the host plus its microbiome to the status of an organism. As we have discussed above, this is a narrow view of the actual complex nature of selection acting on host-microbiome communities.

One of us (A.E.D.) believes that there is already an excessive proliferation of terms in the symbiosis field and that “hologenome” is inaccurately applied to host-microbiome systems, but no substitute term is required. One of us (J.H.W.) believes that there is a need for a useful, more general moniker to describe the

host-microbiome and its associated genomes, without presupposition that the system is evolving as an “individual.” For those desiring an alternate, more general term, J.H.W. suggests “symbiome” for the host-microbiome community (75) and “symgenome” for their combined genomes. Although not as mellifluous as holobiont and hologenome, these terms capture the essence that host and microbiome occur together in an intimate association and do not presuppose that the community is evolving as a unit.

Overview. The concept that biological entities can become organized into larger units of selection has a long history in biology. The groundbreaking book *The Major Transitions in Biology* (49) makes a convincing argument that most of the major biological transitions have involved the evolution of mechanisms that reduce conflict and competition at one biological level, facilitating the evolution of a higher biological level. Examples include the evolution of chromosomes, eukaryotic cells with nuclear and mitochondrial genomes, multicellular organisms, and insect eusociality. These ideas have been developed further recently (48, 76). Therefore, the question of whether host-microbe systems could evolve into units of higher biological organization is reasonable. In fact, two clear cases are the microbial ancestors of mitochondria and chloroplasts that evolved into organelles. However, it must be noted that these events are rare, and the rare examples involve individual host-symbionts becoming integrated into a “hologenome,” not entire microbiomes. Furthermore, a more sophisticated understanding of these systems comes from considering how selection acts at different potential levels of biological organization, including gene, individual, population, species (clade), and ecological community. Thus, evolution of individual organisms has not eliminated genetic conflicts within genomes, which occur when genetic elements have discordant patterns of transmission to future generations (77). The evolution of eukaryotes with nuclear and organelle genomes comes closest to a “hologenome,” but a more complete understanding emerges when it is realized that discordant inheritance patterns of nuclear and organelle genes lead to genetic conflicts and antagonistic coevolution over sex determination.

We do not argue that selection cannot act on the host-microbiome as a unit. We simply argue that evidence for this is weak, and the conditions necessary for it to occur are unlikely. Selection on the joint host-microbiome is just one aspect of the selective universe experienced by hosts and individual microbial associates. The best predictor of whether an individual microbe and host evolve mutualistic “coadaptations” is whether specific host and microbial genotypes cooccur with high fidelity. This is achieved either by a substantial rate of cotransmission (e.g., vertical inheritance) or by strong mutual partner choice (for infectious and environmentally acquired microbes). Such high-fidelity associations are unlikely to occur across the entire microbiome of a host, and where it does not, there are considerable opportunities for complex interactions and effects, only some of which will reflect mutually beneficial coadaptation or unidirectional adaptation. Diverse transmission modes, metapopulation structures, and styles of interaction are the norms in complex host-microbiome assemblages. Therefore, it is highly unlikely that the entire microbiome will evolve as a “holobiont” with its host.

In this article, we have argued that the hologenome concept is unhelpful to the study of host interactions with resident microorganisms because it focuses on one level of selection (the

holobiont), and as a result it is concerned with cooperative and integrative features of host-microbe systems to the exclusion of other kinds of interactions, including antagonism among microorganisms and conflicts between host and microbial partners. In contrast, the intellectual tools provided by the well-established disciplines of ecology, genetics, and evolution provide a rich and effective conceptual framework for analysis of host-microbiome systems. This is not to suggest that the research challenges posed by the microbiome revolution are trivial. Quite the reverse is true: the complexities of these systems demand a strong interdisciplinary perspective.

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