Getting the Hologenome Concept Right:

An Eco-Evolutionary Framework for Hosts and Their Microbiomes

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Abstract

Given the recently appreciated complexity of symbioses among hosts and their microbes, significant rethinking in biology is occurring today. Scientists and philosophers are asking questions at new biological levels of hierarchical organization - What is a holobiont and hologenome? When should this vocabulary and associated concepts apply? Are these points of view a null hypothesis for host-microbe systems or limited to a certain spectrum of symbiotic interactions such as host-microbial coevolution? Legitimate questions, advancements and revisions are warranted at this nascent stage of the field. However, a productive and meaningful discourse can only commence when skeptics and proponents alike use the same definitions and constructs. For instance, critiquing the hologenome concept is not synonymous with critiquing coevolution, and arguing that an entity is not necessarily the primary unit of selection is not synonymous with arguing that it is not a unit of selection in general. Here, we succinctly deconstruct and clarify these recent misconceptions. Holobionts (hosts and their microbes) and hologenomes (all genomes of the holobiont) are multipartite entities that result from ecological, evolutionary and genetic processes. They are not restricted to one special process but constitute a wider vocabulary and framework for host biology in light of the microbiome. We invite the community to consider these new perspectives in biology.

52 **Main Text**

53 The holobiont is a term used to describe an individual host and its microbial
54 community, including viruses and cellular microorganisms [1-6] (Figure 1). It is derived from
55 the Greek word *holos* which means whole or entire. Microbial symbionts can be constant
56 or inconstant and can interact with a host in a context-dependent manner as harmful,
57 harmless or helpful. In most cases, the net outcome of these interspecies relationships
58 often varies with the presence of other symbionts. The term holobiont distinguishes itself
59 by not only recognizing hosts and their obligate symbionts, but also emphasizing the
60 diverse and complex array of symbionts and their dynamic associations within a host. In
61 contrast to binary host-microbial interactions, the emergent properties of complex
62 microbial communities and their hosts are newly appreciated and potentially universal.
63 The host and microbial genomes of a holobiont are collectively defined as its hologenome
64 [1,2], and the pluralistic attributes of a holobiont scale directly to the hologenome (Figure 1).
65 This pluralism is an important point that is frequently missed. Microbial genomes can be
66 stable or labile components of the hologenome, vertically or horizontally transmitted, and
67 the functional traits that they encode are context dependent and may result in damage,
68 benefit, or indifference to the holobiont [7].

69 The hologenome concept of evolution proposes that evolutionary forces acting at
70 the phenotypic level of an individual organism (e.g., a single plant or animal) are instead
71 acting at the holobiont level in many cases. For a given plant or animal, phenotype and
72 fitness can often be deeply dependent on its microbial community [8], in both cooperative
73 and competitive manners. Hologenomic evolution is a relatively new concept and is
74 therefore liable to be interpreted in ways that misrepresent its original conception.

Importantly, a recent paper used different definitions for the holobiont and hologenome, and restricted the term hologenome to those situations when the host and microbes coevolve to form a primary unit of selection [9]. The result is a "straw man" conception of the holobiont and hologenome. The hologenome concept is relatively new to biology and thus requires critical evaluation; but to have a robust debate, skeptics and proponents must use the same terminology. Our central goals in this brief essay are (a) to highlight errors in these recent narrow definitions of the holobiont and hologenome, keeping them consistent with their original pluralistic definitions, (b) to encourage productive discussion and critical inquiry, and (c) to stimulate new ideas and understanding considering the link between phenotype and genotype.

The first argument proposed by skeptics is that if X did not coevolve *sensu stricto* with Y, then the hologenome is not real [9-11]. In this case, X and Y are respectively a microbe/microbial community and a host. However, this argument is not relevant to the hologenome concept. Hologenome is a term that encompasses all of the genomes of the host and its microbes at a given point in time. How the symbiotic community was assembled is independent of how selection can act upon the holobiont. Thus, holobionts can be formed through neutral processes, selection at the level of the host, symbiont or both (Figure 1). Although a component of it, coevolution is not the sole feature of the hologenome and its associated concepts. By way of caricatural illustration, one would not similarly say that if genes X and Y did not coevolve in a host, then they are not part of the genome. Evolution of genomes and hologenomes is not a monolithic process. Genetic conflict, epistasis, selection, drift, etc. are all operational [1,2]. Thus, objections to the

hologenome concept based on a lack of coevolution misrepresent what constitutes a hologenome. To put it simply, coevolution is a process; the hologenome is an entity.

It was recently suggested that a non-coevolutionary application of the word hologenome "would make it sufficiently general that it can be interpreted in any number of ways" [9]. This comment refers to the original and more generally accepted definition of the hologenome as all of the genomes in the holobiont, all of which in turn are evolving in that context [6]. However, using this logic, the word genome would be as unhelpful to biology as the term hologenome because it would be an insufficiently general definition of the types of evolutionary processes occurring within the genome. As noted above, coevolution, genetic conflict, selection, and drift all occur in the genome and hologenome. For this reason, genetic interactions or epistasis between genes in the same genome follow a conceptual continuum to intergenomic and interspecies interactions underlying community phenotypes in hologenomes [1,12].

Prevalent misuse of coevolution to explain host-microbe associations was the impetus for some of us coining the word “phylosymbiosis” [13]. It describes the concordance between a host phylogeny and microbial community dendrogram that depicts community relationships based on the degree of shared taxonomy and/or abundance of members of the community [14-17]. Phylosymbiosis does not *a priori* imply coevolution or codiversification to the observed pattern of symbiotic microbial community relationships recapitulating host phylogenetics. Moreover, it "does not presume that microbial communities are stable or even vertically transmitted from generation to generation" [15,16]. Rather, it refers to a pattern in which changes in separate parts of the holobiont (host genome and microbiota) are related in a concordant manner across lineages of holobionts.

It is also a stepping-stone from population genetics to community genetics because when phylosymbiosis is observed under strictly controlled conditions, it tests whether variation in holobiont assembly is primarily stochastic or deterministic [13,15,18]. Stochastic assembly means that each microbe has an equal opportunity of colonizing a host. Deterministic assembly reflects ecological selection of a particular non-random microbial community and its host, without reference to which partner, or potentially both, is doing the selection, and it can be affected by genetic variation in the host or microbial species. In this context, when genetic variation in the interacting species affects community assembly, it has been defined as broad sense "community heritability," or H^2_C [19,20]. H^2_C measures a "heritable basis to trophic-level interactions" [21]. Controlled studies of microbial community assembly across different species of *Nasonia* wasps and *Hydra* have yielded such phylosymbiotic patterns [13,14]. If there is a significant H^2_C, natural selection can act on ecological communities [18], including organization of the holobiont and its emergent phenotypes [20].

Discussion of evolutionary processes brings forth a second unfounded argument against the hologenome concept, namely that holobionts and their hologenomes must be the "primary" unit of selection for the concept to be informative [9]. This strict claim leads biologists into error, as multiple levels of selection can operate simultaneously. For example, selfish genetic elements can be selected within a genome that is in turn selected for any number of phenotypes that affect fitness—this is uncontroversial. While the holobiont is posited to be "a unit of selection in evolution" [2,22-24], it is critically not proposed as the only or primary unit of selection [1,2]. Primariness varies with what traits are targeted by natural selection. From a phenotypic perspective, selection works on trait variation that influences which entities reproduce and in what relative proportions they do so. Given this

143 tenet and the impact of microbes on host phenotypes, the target of selection at the so-called
144 host level will in many phenotypic cases be the holobiont—the extent to which is yet to be
145 determined. What we wish to emphasize here is that the holobiont does not imply group
146 level selection. Rather, variation in the hologenome yields variation in holobiont
147 phenotypes upon which various evolutionary (and ecological) processes at different levels
148 can act. The evolutionary response to selection can result in changes in the genetic
149 composition of the host genome, microbial genomes or both. We believe this concept
150 provides new perspectives.

151 As we have emphasized in different venues, it is also true that just as large parts of
152 the nuclear genome can evolve neutrally or be in conflict, so too can large parts of the
153 hologenome [1,2]. For example, "hologenomic drift can occur at all the different levels of the
154 holobiont from single genes of the microbes or the host to the holobiont itself" [2]. We would
155 be remiss to not be critical of our own inconsistent statements about the relative roles of
156 cooperation and conflict in hologenomic evolution. In *The Hologenome Concept*, some of us
157 stated that "evolution of animals and plants was driven primarily by natural selection for
158 cooperation between and with microorganisms" [2] while in other venues the concept
159 "places as much emphasis on cooperation as on competition" [25]. This latter statement is
160 more precisely aligned with the pluralistic nature of the holobiont, namely that "natural
161 selection...on holobiont phenotypes...can work to remove deleterious nuclear mutations or
162 microbes while spreading advantageous nuclear mutations or microbes" [1]. In fact, some of
163 us argued that conflicts of interests resulting from the nature of the transmission of
164 microbes to the next host could select for microbes that can manipulate the biology of their
165 host to improve their own transmission [26]. The holobiont is not a conglomerate that arises

solely from cooperation. Rather, it is a hierarchical level that can supersede the individual host and encompass the organismal, genetic and phenotypic features of the host in association with its microbial community, incorporating both competitive and cooperative selective systems [27]. Hologenomes then exist as hierarchically nested levels of genomes, in which all levels of selection are potentially in play.

In summary, we anticipate that many subdisciplines in biology will benefit from a theoretical and experimental framework that broadly encompasses the ecology of holobionts and evolution of hologenomes. The hologenome concept is a comprehensive and relevant eco-evolutionary framework for which critical questions remain. For example, can a response to selection on host traits be driven solely by changes in the genomes and/or membership of a host-associated microbial community? How taxonomically widespread is phylosymbiosis? What is the strength of selection on holobiotic interactions required to maintain consistent association with environmentally-acquired microbes each generation? How does selection operate on community phenotypes if H^2_C is variable throughout the holobiont's lifetime due to the lability of microbial communities? Can the lability of microbial communities explain inter-individual variations and help maintain a higher phenotypic diversity within a holobiont population? Evolution of the hologenome refers to the genetic basis of eco-evolutionary processes underlying community phenotypes of the holobiont. We suggest that the null framework for the newly appreciated complexities in the host-microbe consortia and their genomes is best reflected by the terms holobiont and hologenome and their associated concepts.

187 **Figure Legend**

188 Figure 1. Holobionts are entities comprised of the host and all of its symbiotic microbes,
189 including those which affect the holobiont's phenotype and have coevolved with the host
190 (blue), those which affect the holobiont's phenotype but have not coevolved with the host
191 (red), and those which do not affect the holobiont's phenotype at all (gray). Microbes may
192 be transmitted vertically or horizontally, acquired from the environment, and can be
193 constant or inconstant in the host. Therefore, holobiont phenotypes can change in time and
194 space as microbes come into and out of the holobiont. Microbes in the environment are not
195 part of the holobiont (white). Hologenomes then encompass the genomes of the host and
196 all of its microbes at any given time point, with individual genomes and genes falling into
197 the same three functional categories of blue, red and gray. Holobionts and hologenomes are
198 entities, whereas coevolution or host-symbiont interactions are processes.

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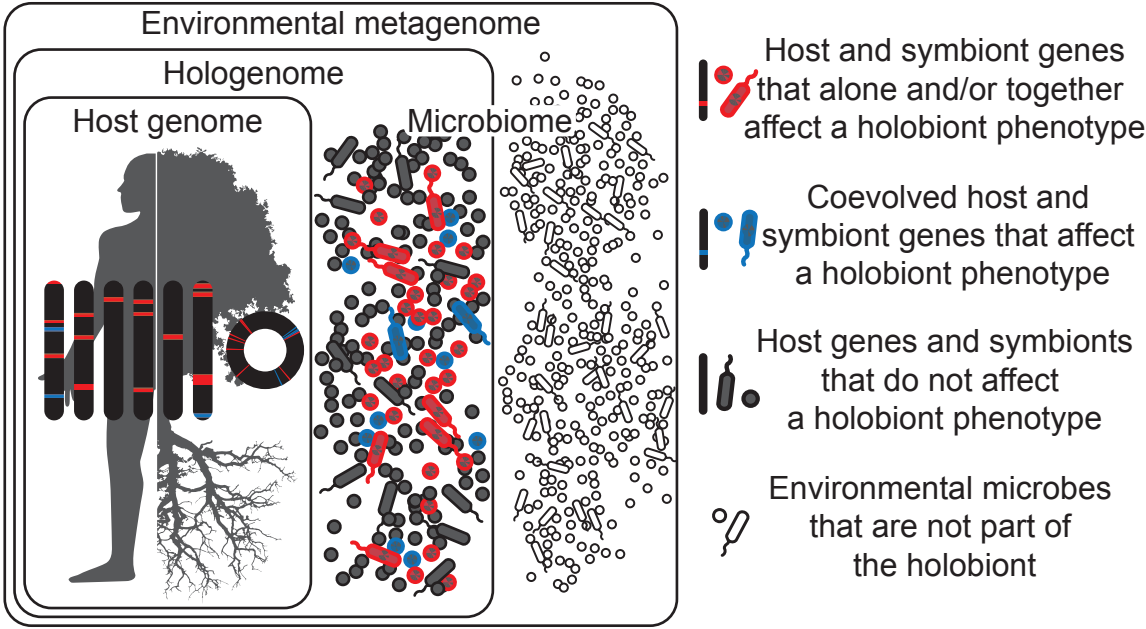
Environmental metagenome
Hologenome
Host genome
Microbiome
Host and symbiont genes that alone and/or together affect a holobiont phenotype
Coevolved host and symbiont genes that affect a holobiont phenotype
Host genes and symbionts that do not affect a holobiont phenotype
Environmental microbes that are not part of the holobiont