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The hologenome concept: we need to incorporate function

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Abstract Are we in the midst of a paradigm change in biology and have animals and plants lost their individuality, i.e., are even so-called 'typical' organisms no longer organisms in their own right? Is the study of the holobiont—host plus its symbiotic microorganisms—no longer optional, but rather an obligatory path that must be taken for a comprehensive understanding of the ecology and evolution of the individual components that make up a holobiont? Or are associated microbes merely a component of their host's environment, and the holobiont concept is just a beautiful idea that does not add much or anything to our understanding of evolution? This article explores different aspects of the concept of the holobiont. We focus on the aspect of functional integration, a central holobiont property, which is only rarely considered thoroughly. We conclude that the holobiont comes in degrees, i.e., we regard the property of being a holobiont as a continuous trait that we term *holobiontness*, and that holobiontness is differentiated in several dimensions. Although the holobiont represents yet another level of selection (different from classical individual or group selection because it acts on a system that is composed of multiple species), it depends on the grade of functional integration whether or not the holobiont concept helps to cast light on the various degrees of interactions between symbiotic partners.

Keywords Holobiont · Hologenome · Symbiosis · Evolution · Function · Functional integration

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Introduction

Many examples have been reported where the association between a host and its symbiont(s) generates a phenotype that the single components would be unable to produce. For example, the Hawaiian bobtail squid glows at night as a result of its association with the microbial symbiont *Aliivibrio fischeri*. The generation and the architecture of the squid's light organ, where the increased bacterial concentration activates the vibrio's quorum-sensing based production of blue-green light (Montgomery and McFall-Ngai 1994), are the result of a fascinating exchange of molecular signals between the squid and the endosymbiont (Chun et al. 2008; Kremer et al. 2013; Lamarcq and McFall-Ngai 1998; McFall-Ngai 2014a, b; Nyholm et al. 2000).

The concept of a holobiont describes a long-term physical association between two or more organisms that are members of different species through a significant portion of their life history (Margulis 2003; Mindell 1992). The relationship between the partners could be: (1) spatial, when (one of the) partners require physical contact in most

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or part of their life history; (2) metabolic, when a product of the metabolism of one partner becomes a nutrient for the other(s) (e.g., symbiotrophy); (3) genetic, when a gene or a gene-product is transferred between the partners. In addition, any of these relationships may be either permanent or temporal. In a recent publication, the holobiont was defined even less specifically "as an individual host and its microbial community including viruses and cellular microorganisms" (Theis et al. 2016).

The genetic relationship takes prominence when we consider the hologenome concept of evolution. This concept specifies that the host nuclear genome, mitochondrial and, where it applies, plastid genome, and microbiome are an ensemble of genes that together form a unit of natural selection (Godfrey-Smith 2009). Each unit of selection is considered a biological individual, so that also so-called atypical organisms like *Physalia physalis* and other siphonophores count as individuals (Hull 1980). So a hologenome is, by definition, the genome of a biological individual, while the host, which forms a joint unit of selection with its microbiota, loses its status as an individual.

The hologenome is defined as the sum of the genomes of the host and its microbiota. Hence, the evolution of the hologenome can occur by changes in the host genome (nuclear, mitochondrial or plastidal) and/or in any of the associated microbial genomes, and relies on cooperation and conflict between the genomes within the holobiont, as much as on competition with other holobionts (Zilber-Rosenberg and Rosenberg 2008).

Fundamentally, if hologenomes are heritable and produce variable holobiont phenotypes, these could compete with each other for survival and reproduction and selection could act at the level of the holobiont. Selection might then be able to drive changes in any component of the holobiont, based on its effect on the phenotype. These components could either be the host genes, the composition of the microbiome or microbial genes, or any combination. In that respect, one could calculate—analogous to narrow-sense heritability—the degree of heritability for any holobiont phenotype using a parent–offspring correlation (van Opstal and Bordenstein 2015).

The holobiont concept is fascinating, for sure. However, do we really need this concept? Does it allow asking or answering questions that were otherwise inaccessible or intractable? Objections might be based on various arguments concerning different explanatory goals (Leggat et al. 2007; Moran and Sloan 2015).

1. Is the holobiont concept as unambiguous as it may seem? Is it sufficiently precise to figure in scientific explanations without blurring them? Proponents of the holobiont theory maintain that the holobiont's microbiome includes, among others, microbes that do not affect the holobiont's phenotype (Theis et al. 2016). If affecting the phenotype is not required for a microbe to count as part of the holobiont, one is left to wonder whether and how a line between the holobiont and microbes in its external surroundings can be drawn. Additionally, the holobiont theory of evolution proposes that not only the alteration of the microbial community by amplification and horizontal gene transfer, but also the novel acquisition of microbes from the environment provide mechanisms for rapid molecular and phenotypic evolution including speciation. These dynamics inevitably make the biological *object* holobiont hard to define and, thus, to study. Furthermore, they hint at the often-underplayed or neglected existence of distinct degrees of integration between the components of a biological whole.

2. Does the holobiont concept help us to understand the evolution of host–symbiont associations? As an alternative view, symbionts might be considered simply as one component of the hosts' environment, not really different from other biotic or abiotic components of the environment, such as predators, parasites or the availability of nitrogen or phosphorus. Additionally, the concept of co-evolution can already explain reciprocal adaptations in host–symbiont systems and focuses on the proximate mechanisms and evolutionary consequences of organismal interactions, just as the holobiont concept does. Thus, what would a shift to the holobiont/ hologenome help us to elucidate that co-evolution could not already explain, or maybe how would we argue differently from a holobiont's perspective?

3. And last, do we need this concept to explain the evolution of organisms? For all we know at the moment, many host (microbial) phenotypes are dependent on the host (microbial) genotype, being little or not affected by the interactions between host and microbiota. Hence, it is not always necessary to invoke the holobiont concept to understand the phenotype or evolution of a host or the associated microbe trait, i.e., it might be irrelevant for the understanding of the evolution of wing venation or wing coloration in *Drosophila* or the arrangement of the cilia on an endosymbiont.

Many of the above-cited objections have been discussed in previous studies, and several arguments have been brought forward against (Moran and Sloan 2015) or in support of (Bordenstein and Theis 2015; Theis et al. 2016) the concepts of holobiont and hologenome. In our manuscript, we demonstrate that current debates on these concepts are based on a problematic view of functional integration (wholeness). We begin by pointing out that a holobiont cannot be unambiguously defined nor can

boundaries between different kinds of holobionts be described. Next, we introduce two distinct concepts of functional integration and describe how different concepts of function lead to different views of what is "whole" and by extension a holobiont. Our manuscript underlines that in order to further current understanding of the properties of biological systems, it is necessary (1) to either arrive at a shared definition of functionality, or (2) that claims about functionality are only made relative to the concept of function that is being applied.

Dimensions of the holobiont

Candidates for holobionts range from classical systems of host and mutualist symbionts like plant and mycorrhizal fungi, or endosymbiotic systems like the ciliate *Paramecium* with *Rickettsia*, to more loosely connected systems such as mammals and their gut bacteria. Some of those symbioses might be obligatory, others facultative or even random. The plethora of examples (some are illustrated in Table 1) virtually provides us with a continuum of degrees of interaction within genetically heterogeneous biological units. We, therefore, do not consider it helpful to try drawing, at the present state of research, a sharp line between holobionts and more loosely assembled associations. It seems instead more fruitful to employ the concept of the holobiont as a model or as an epistemic *tool* that allows highlighting the integrative aspect of symbioses of

different kind. To sharpen this tool, we will increase the pool of examples and include two more exotic symbiotic systems than are usually considered in the current literature on holobionts, lichen and *Chlorochromatium aggregatum*. Lichens consist of at least two very different organisms: a heterotrophic fungus (mycobiont) and a photosynthetic organism (photobiont), which can either be a cyanobacterium (cyanobionts) or a unicellular green alga (phycobiont) (Nash 2008). The phototrophic consortium *Chlorochromatium aggregatum*, on the other hand, is "*a symbiosis that may represent the highest degree of mutual interdependence between two unrelated bacteria not associated with a eukaryotic host*" (Liu et al. 2013). *C. aggregatum* is a motile photosynthetic community in which symbionts (the green sulfur bacterium *Chlorobium chlorochromatii*) live on the surface of their partner, a beta-proteobacterium. The non-motile photosynthetic epibionts depend on the beta-proteobacterium for movement (Frostl and Overmann 1998), whereas the flagellated, non-pigmented, heterotrophic beta-proteobacterium depends on the epibionts for energy (Cerqueda-Garcia et al. 2014). Widening the scope in this way helps to establish an important, yet often missing, further dimension of integration, namely *functional* integration, besides the usually stressed spatial, metabolic, and genetic integration of a holobiont. We will start filling the gap and scrutinize the functional integration of the holobiont. For one, we will show that, as all dimensions of a holobiont, functional integration comes in degrees.

Table 1 Examples of partnerships with some of their principal characteristics

	Host	Symbiont	Physical proximity	Co-inheritance	Functional integration
A	Eukaryote	Mitochondrium	Intracellular	Maternal transmission	Fatty acid oxidation, Krebs cycle, and oxidative phosphorylation
B	Eukaryote	*Wolbachia*	Intracellular	Maternal transmission	Host manipulation (feminization, parthenogenesis)
C	Human	Gut bacteria (*Escherichia coli*)	Inside body lumen	Acquisition from the mother during birth or from the food	Modulation of metabolic phenotypes
D	Coral	Dinophyceae (*zooxanthellae*)	Intercellular	Acquisition from the parental colony or through ingestion	Exchange of metabolites in oligotrophic environment. Loss of association results in bleaching and death
E	*Paramecium*	*Chlorella*	Intracellular	Acquisition through ingestion	Facultative mutualistic trophic interactions. Host cells retain the ability to grow in the absence of the symbiont
F	Fungus	Cyanobacteria or Chlorophyceae	Intercellular	Vertical or horizontal transmission of the photobiont	Structural integration and trophic interactions. Association allows colonization of extreme ecological niches
G	Eukaryote	Cutaneous microbiota	On body surface	Acquisition from the mother during birth and from the environment	Protection

A (Margulis 1970), *B* (O'Neill et al. 1992; Rousset et al. 1992; Stouthamer et al. 1993), *C* (Finegold et al. 1983), *D* (van Oppen 2004), *E* (Karakashsj et al. 1968), *F* (DePriest 2004), *G* (Grice and Segre 2011)

Holobionts: what kinds of wholes?

Biological wholes, be they organisms or holobionts, are functionally integrated. Their components interact and subsets of them jointly bring about the capacities of the whole. Most components may have one or many functions, they may contribute to one or many capacities, and most capacities are tuned in some way or another with other capacities. This holds for metabolic pathways or networks as well as for sensory input or for mechanical contributions to locomotion (Gilbert et al. 2015; McFall-Ngai et al. 2013). We consider integration of this kind as a relevant aspect of being a biological whole. Being a whole is not just a matter of morphological closure or spatial continuity. Being a whole, in the realm of biology, is being constituted by components that interact in a way that the functional contributions to the system complement each other (von Bertalanffy 1968; Mossio et al. 2009). What counts as a biological whole, thus, depends on the concept of function applied. Since there is a plethora of such concepts, and since neither biologists nor philosophers of biology restrict themselves to using only one concept (Krohs 2009), we will discuss two alternatives. The two concepts of function

we are using (Box) support differing views of functional integration and of biological wholeness (Fig. 1).

(i) According to the **systemic concept of function**, any parts of a system that contribute jointly to a capacity of the system are functionally integrated (Cummins 1975). Persistence of the system is brought about by some of those functional contributions being self-regulatory. Systemic wholes thus are complex entities that persist through time due to self-regulatory interactions of their components. Systemic biological wholes, therefore, need not be the result of a process of natural selection. They may also emerge newly from spontaneous or even accidental interaction. To which degree a systemic biological whole is a unit of selection depends on the strength and persistence of the interaction.

(ii) In contrast to the systemic concept, the **etiological concept of function** focuses on the causal history of the presence of a component in a system. According to this concept, only those parts of a system that were selected to contribute to a capacity of the system have the function to contribute in this way (Millikan 1984; Neander 1991). Mere contribution, without being selected for this contribution, does not suffice. At least some of those functional
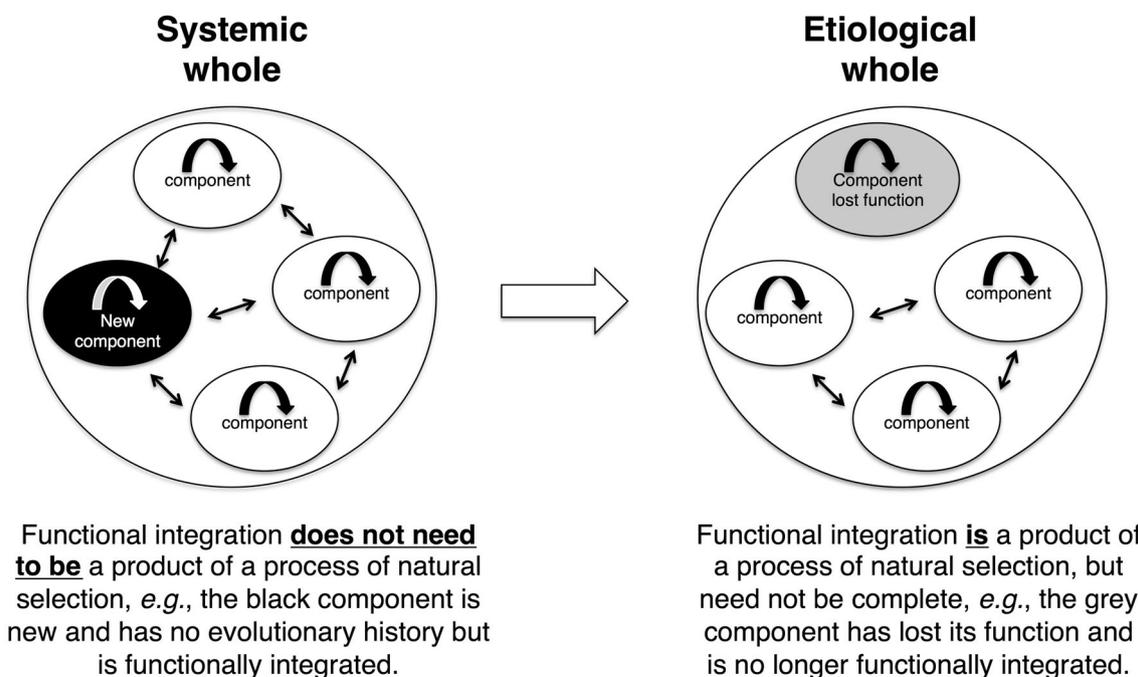

Systemic whole

Functional integration **does not need to be** a product of a process of natural selection, *e.g.*, the black component is new and has no evolutionary history but is functionally integrated.

Etiological whole

Functional integration **is** a product of a process of natural selection, but need not be complete, *e.g.*, the grey component has lost its function and is no longer functionally integrated.

Fig. 1 Systemic and etiological wholes. Systemic wholes are complex entities, which persist through time due to the interactions of their components, which are in part self-regulatory. Etiological wholes are complex entities which were selected due to their being systemic wholes. Components of an etiological whole need no longer be fully or at all integrated systemically, though they were during earlier stages of evolution (as indicated by incomplete or missing connectors). Components that are functionally isolated in an adult

organism might have had a systemic function during development of the organism for which they were selected, e.g., as scaffolds (Griesemer 2014). So a transition from a systemic to an etiological whole might even occur during ontogenesis. Other scaffolds like external developmental resources might be systemically integrated (Minelli 2016) without becoming a component of an etiological whole

contributions were selected because they contributed to self-regulation. Some of the functions, however, might no longer be performed by the respective components. Etiological wholes, therefore, may contain functions—or function bearers—which are no longer fully integrated, but which belong to the actual whole owed to its selection history. The criterion for being an etiological whole, thus, is being the product of the process of natural selection.

At first glance, it may seem as if the difference between systemic and etiological wholes is just one of perspective: the focus being on the system properties in the first case, on its generation in the second. But different things will count as wholes in the two cases. On the one hand, an association of a host with a symbiont may well have the status of a systemic whole if it shows capacities to which both partners contribute. This holds even in cases where the partners do not have a shared selective history and, thus, the holobiont partners do not have the status of an etiological whole. A new association between a fungus and an alga, or a mammal and a bacterium (which happens to make it into the gut of the mammal, but which never interacted evolutionarily with it), may thus count as holobionts in the systemic sense. On the other hand, an evolutionary stable former symbiosis in which the symbiont does no longer contribute to any capacity of the host and is thus no longer systemically integrated may nevertheless count as an etiological whole and thus be a candidate for a holobiont in the etiological sense, despite the lack of present functional integration.

Functional systemic wholes may acquire the additional status of etiological wholes when stabilized through a common selective history. An etiological whole may lose possible initial systemic wholeness when part of the functional integration is lost. Most important for our present purpose, however, is the more general point that the ascription of wholeness depends on the concept of function applied. Relative to the explanatory goals, one may apply one or the other concept of function and, consequently, focus on systemic or on etiological wholes. However, this classificatory approach remains open for amendments: While the two aforementioned concepts of function constitute the most widely discussed approaches, other explications of the concept of function have been proposed, in particular the organizational account (Mossio et al. 2009; Saborido et al. 2011) and the type fixation account (Krohs 2009, 2011). Since we are interested in the epistemic role of the application of the concept of holobiontism rather than in developing a detailed empirical landscape of single cases, we stick with the two classical concepts of function. It is important to notice that some organismal associations are holobionts according to one and/or another explication of the concept of function and that some associations are not holobionts at all.

Box Two concepts of function

A function of a component is first and foremost the role it fulfills within a system or its contribution to a capacity of the system (Cummins 1975). These roles are called **systemic functions**. Any component—be it a molecule or molecular structure, an organelle, an organ, or a symbiont—may have several systemic functions. According to this systemic concept, a component ceases to have a particular function if it has no longer the disposition to perform it.

Sometimes and in some contexts, biologists want to speak about functions in a stronger sense: not as actual, perhaps accidental roles or dispositions, but as the roles a component was selected for. Since functions in this sense were fixed during the causal history of the trait, in its etiology, they are called **etiological functions** (Millikan 1984; Neander 1991). Etiological functions are often said to be selected effects or selected systemic functions. Kidneys, for example, were selected for filtering the blood (via selection of the organisms they were components of), so filtering blood is their etiological function.

Hologenomes: insights from the degrees of functional integration within holobionts

The extent of functional integration within genetically heterogeneous systems may vary greatly between biological wholes. When genetic material is translocated from a symbiont or endosymbiont to the host, we see functional integration at the level of jointly enabled metabolic processes. Integration and wholeness in these cases are etiological. An example is the case of mitochondria and host eukaryotic cells. It is long accepted that mitochondria represent anciently acquired prokaryotes that have a fully integrated partnership with the eukaryotic genome. It takes more than 1300 nuclear encoded genes to make functional human mitochondria (Wallace 2005). Nowadays, we do not recognize the eukaryotic cell as a holobiont (e.g., mitochondria of humans do not have a different taxonomic name), since it is not possible to separate mitochondria and host cell, and it is easy to imagine that in the future other holobionts will be recognized similarly as only one organism that is the target of selection and adapts to novel environments (Guerrero et al. 2013). Functional integration of this kind may also be present at the level of gene regulatory networks (GRN), like in the developmental GRN of sea urchins (Peter and Davidson 2010), which includes mitochondrial and nuclear genes alike. In such cases of etiological wholeness, we may straightforwardly classify the sum of both genomes as a hologenome. Noteworthy, even in this extremely old system (eukaryotes evolved

>1 bya) conflicts between the two seemingly completely integrated partners can emerge under a variety of evolutionary scenarios (Cosmides and Tooby 1981).

The matter becomes more complicated when the holobiont in question is a systemic whole, where the symbiotic partners show systemic integration without forming a unit of selection. Let us take gut bacteria and the mammalian host as an example. Functional integration might occur, but much more indirect or at a higher level than that of a GRN. For example, bacteria produce vitamins and essential amino acids and, thus, contribute many capacities of the holobiont, but most of them could be replaced by other strains or species of bacteria and it is all but clear that exactly this symbiosis has a shared and uninterrupted selective history (Moran and Sloan 2015). So we may tend calling the sum of mammalian genome plus microbiome a hologenome on the basis of a systemic concept of wholeness, but not on an etiological one. In sum, together with the concept of a holobiont, also that of the hologenome varies with the concept of function applied, via a varying concept of wholeness.

Degrees of functional integration and mode of symbiont transmission

Because vertical transmission increases the efficiency of response to selection, it is commonly thought that holobionts may only form a unit of selection when the associated microbe/microbiome is co-inherited or vertically transmitted. Symbiont vertical co-transmission is not a prerequisite of etiological wholes however. Animal or plant hosts may laterally acquire symbiont(s) and, together, generate phenotypes, which not only dissolve in the absence of either of the two components, but also increase the fitness of the organism/holobiont (Rosenberg and Zilber-Rosenberg 2013). This is the case of the leguminous plants, which acquire rhizobia from the soil (Heath and Tiffin 2007; Jones et al. 2007). In this symbiosis, the plant and the bacterial genes, together, produce leghemoglobin (Ott et al. 2009), a molecule that facilitates the nitrogen-fixing activity of the bacterial enzyme nitrogenase by maintaining the local oxygen concentration at a low level. The presumably selected effects of the leghemoglobin in the legume-rhizobia system indicate that those ancestral rhizobial cells, which contributed to the joint biosynthesis of leghemoglobin and thus showed an efficient nitrogen-fixing activity, had a higher fitness than those that did not contribute. As a result, this trait has persisted over evolutionary time.

Do holobionts come in degrees?

Two conclusions that can be drawn from our discussion thus far are: (1) holobionts exhibit different levels of functional integration, and (2) selection of holobiont phenotypes is compatible with vertical and horizontal symbiont transmission. We attempt to combine and visualize these two aspects in a multi-dimensional space that accounts for the degree of co-inheritance, functional integration, and physical proximity between the components of the partnerships illustrated in Table 1 (Fig. 2). What emerges from this simple visualization—which reminisces the illustration of degrees of reproductive integration and specialization in genetically largely homogeneous organisms and groups presented by Godfrey-Smith (Figure 5.1, p. 95 in Godfrey-Smith 2009)—is a picture of almost

Fig. 2 The multidimensional space of "*holobiontness*". Physical proximity, functional integration, and co-inheritance were selected as three of the several possible criteria (dimensions) to determine the degree of holobiontness of specific cases of interacting organisms (A–G, cf. Table 1) along different axes. The degree of functional integration is indicated by intensity of shade of *gray* (*dark gray* equals high degree and *light gray* low degree of functional integration). Note that the positions of the examples can only be broadly estimated

A: Eukaryote - mitochondrium
B: Eukaryote - *Wolbachia*
C: Human - *E. coli*
D: Coral - algae
E: *Paramecium - Chlorella*
F: Fungus - algae
G: Eukaryote - cutaneous microbiota
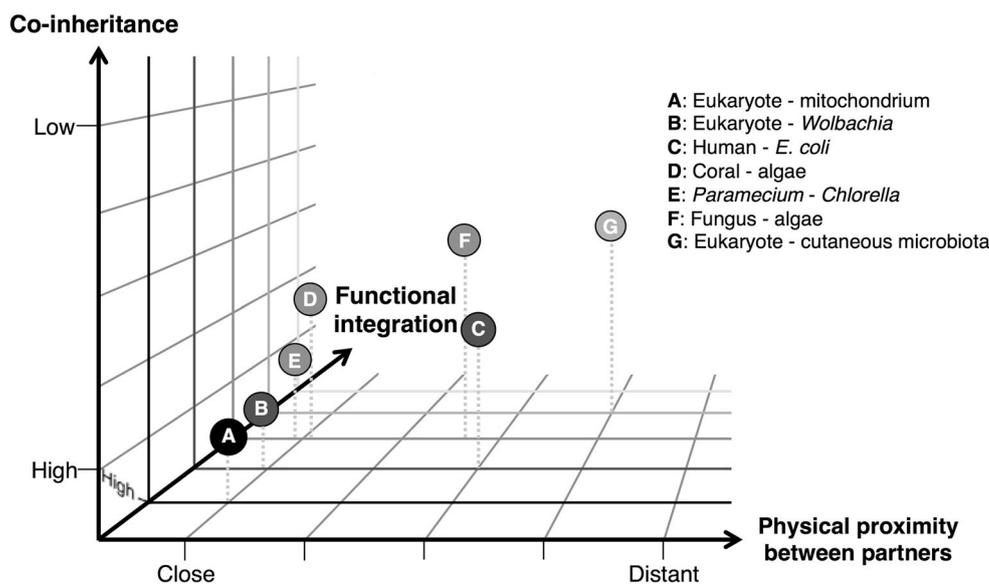
continuous variation in several dimensions that may together describe the degree of being a holobiont, a property that we propose to name *holobiontness*.

Recalling that not all symbiotic associations contribute to fitness (positive or negative), this multi-dimensional space of holobiontness includes the two kinds of wholeness we described above, systemic and etiological, without strictly discerning them. It still remains to be clarified to which degree the class of holobionts of one concept overlaps with the class of those found under the other concept. It also remains an open question whether actual cases of wholeness under each of the paradigms come indeed in degrees of holobiontness, or whether wholeness in nature is not quantitative but rather qualitative. Asking these and similar questions will allow better understanding and classifying complex biological entities. Answering these questions is made possible only by thorough reflections on the dimensions of the concept of a holobiont. Thus, from our perspective, the concept of a holobiont can be viewed as a tool that helps to find similarities among and differences between the multifarious cases of biological complexity that exist in nature.

Does the holobiont concept help us to understand the evolution of host–symbiont associations?

Looking only at the holobiont as a functionally integrated unit of selection might miss important evolutionary dynamics that occur at the level of the participating organisms. (Pre-)adaptations of the host may be crucial for establishment and fate of a symbiosis, e.g., metabolic conditions limiting proliferation of symbionts can keep the host from being overrun by the symbiont (Herren et al. 2014). The host environment may also restrict the distribution of endosymbionts to specialized host cells, e.g., bacteriocytes in aphids (Moran and Baumann 2000), or their proliferation rate (van Ham et al. 2003). On the other hand, certain features of the symbiont, e.g., traits that prevent its replacement by other symbionts, or that avoid its ejection by the host, could stabilize the symbiosis. Directly or indirectly such (pre-)adaptations of the symbiont could be the reason for preventing a strategy change of the host, e.g., going from herbivory to a predatory life style. Intriguingly, such (pre-)adaptations become important regulators of the holobiont as a functionally integrated unit. Some of the dynamics described above are highlighted in the case study presented below.

The *Nasonia–Wolbachia* system

A circumstance in which it might be insufficient to only look at the holobiont, rather than the host and microbiome individually, is the contribution of microbes to speciation or, more specifically, the reduction of gene flow between the host components of a holobiont due to differences in the microbiomes of two diverged holobionts. *Nasonia* is a parasitoid wasp genus that is infected by species-specific *Wolbachia* endosymbionts that cause cytoplasmic incompatibility in hybrids. Once cured from their *Wolbachia* endosymbionts by antibiotic treatment, different *Nasonia* species can produce viable and fertile hybrids (cured strains have been kept in the laboratory for decades and can occasionally be found in nature). However, F_2 hybrid males suffer from severe incompatibilities that are asymmetric depending on who donates the cytoplasm, indicating that the nuclear genome and cytoplasm have accrued additional incompatibilities (Breeuwer and Werren 1995; Gadau et al. 1999; Gibson et al. 2013; Niehuis et al. 2008). Both Brucker and Bordenstein (2013) and Gibson et al. (2013) worked independently on the same F_2 hybrids to determine the cause of this incompatibility. Brucker and Bordenstein (2013) demonstrated that they could rescue the F_2 hybrids (at least till pupation) by rearing them in a germ-free environment, supporting the idea that F_2 hybrid breakdown in *Nasonia* is in part due to the incompatibility of species-specific microbes (independent of *Wolbachia*) and a recombined hybrid nuclear genome that shows significant changes in its expression pattern in comparison to both parental genomes. In contrast, Gibson et al. (2013) found evidence for nuclear–nuclear and nuclear–mitochondrial incompatibilities involving the oxidative phosphorylation complex. Interestingly, the rescued hybrid males from Brucker and Bordenstein still showed a biased recovery for the maternal nuclear genome at several nuclear markers on *Nasonia*'s chromosome 5, indicating that both microbial and genetic incompatibilities are responsible for the observed F_2 male hybrid breakdown and hence both can reduce gene flow. Thus, these studies suggest that we probably have two different mechanisms responsible for the observed F_2 hybrid breakdown in *Nasonia*, one that can be ameliorated to some degree by rearing larvae in a germ-free environment and one that cannot be rescued by antibiotic treatment because it is based on microbiome-independent, genetic incompatibilities. It follows that studying these incompatibilities through the holobiont or hologenome concept only would be misleading. Additionally, it remains unclear whether the rescue effect of antibiotic treatment in *Nasonia* is due to the disruption of co-evolved host–microbe interactions or due to preventing opportunistic bacteria from overrunning a weakened host defense in hybrids (owing to nuclear–nuclear or nuclear-mitochondrial incompatibilities), i.e., whether etiological or systemic integration is at stake.

Analogies between the concepts of holobiont and superorganism

In many respects, the holobiont concept resembles the previously proposed concept of the superorganism (but see Bordenstein and Theis 2015). This latter concept (sensu Hölldobler and Wilson 2009; Reeve and Holldobler 2007) posits that as the members of eusocial colonies become evolutionary and functionally more integrated, the selection/evolutionary conflict between members of the colony is greatly reduced and selection between superorganisms/colonies takes precedence, determining phenotypic evolution of the superorganism, e.g., division of labor, social organization, polymorphism, physiological organization etc. (Reeve and Holldobler 2007). Reeve and Holldobler (2007) also emphasize that not all eusocial species (including ants, bees, and wasps) should be considered superorganisms because in many eusocial species intracolonial (evolutionary) conflicts are still stronger than the selective forces that act at the level of the colony. An example of intracolonial conflicts superseding group level selection is worker reproduction. Workers of most eusocial species have retained the ability to produce males and sometimes compete for reproduction with the queen or other workers. In extreme cases, workers can usurp the position as principal egg layers. In some ponerine ants, like *Harpegnathos saltator*, *Dinoponera* or *Diacamma*, mated workers have superseded queens either completely (a queen morph no longer exists in those species) or regularly during colony development, and there is fierce competition between workers of these colonies to become the principal egg layer (Hölldobler and Wilson 2009; Liebig and Poethke 2004; Peeters 1997). In analogy to the discussions of the superorganism, we propose that the concept of the holobiont is especially useful if the selective forces between its components are reduced relative to the selective forces between holobionts, allowing further refinement and integration of the components of the holobiont.

Conclusions

If every association between a eukaryote and a prokaryote is viewed as a holobiont, then the concept becomes meaningless, because many of these associations might have no holobiont phenotype other than the sum of the phenotypes of the components, or no impact on the evolutionary fate of its components (host and symbiont). On the contrary, entirely disregarding the concept of holobiont (1) underplays those interactions between symbiotic partners, which can *de facto* create variable and predictable holobiont phenotypes on which selection can act, regardless of the mode of symbiont transmission. Moreover, (2) it dismisses the chance to further differentiate types of association. Thus, it seems to us that neither of these extreme views is useful.

Currently, there is no clear line that delineates "holobiont" and "non-holobiont", no clear-cut criterion that distinguishes between both cases. In our opinion, holobiontness comes in degrees and is differentiated in several dimensions. Whether a symbiosis is regarded as a holobiont, or multiple genomes within a biological whole as a hologenome, depends on the concept of functional integration applied, and on the degrees of functional, genetic, and spatial integration. More generally, it depends on the perspective and on particular research questions.

The concept of the holobiont becomes highly valuable when used to discern aspects and differentiate degrees of integration between symbiotic partners. This way, the concept of holobiont becomes an epistemic tool that helps to acknowledge and to classify the various levels of integration within and between organisms. It allows detaching, e.g., the question of whether an association is co-inherited (vertical transmission) or re-established in a new generation (horizontal transmission) from the question of whether an association is a unit or a target of selection. Holobiontness is compatible with any positive answer on at least one of those questions, but, depending on the answer, holobiontness will have a special twist. Knowing the twist is much more informative than reserving the term for exactly one form of integration or asking whether some association matches the very demands of such a strict, arbitrarily delineated concept.

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