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Title: Holobionts and the Ecology of Organisms – Multi-Species Communities or Integrated Individuals?

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Abstract:

It is now widely accepted that microorganisms play many important roles in the lives of plants and animals. Every macroorganism has been shaped in some way by microorganisms. The recognition of the ubiquity and importance of microorganisms has led some to argue for a revolution in how we understand biological individuality and the primary units of natural selection. The term “holobiont” was introduced as a name for the biological unit made up by a host and all of its associated microorganisms, and much of this new debate about biological individuality has focused on whether holobionts are integrated individuals or communities. In this paper, I show how parts of the holobiont can span both characterizations. I argue that most holobionts share more affinities with communities than they do with organisms, and that, except for maybe in rare cases, holobionts *do not* meet the criteria for being organisms, evolutionary individuals, or units of selection.

Keywords: holobiont, symbiosis, individuality, organism, bacteria, microbe

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Introduction

Multicellular organisms have been engaged in symbiotic relationships with microorganisms throughout their evolutionary history (Moran 2006). It was long thought that all macroorganisms are routinely colonized by a large number of microorganisms, but the details and extent of macrobe-microbe interactions remained difficult to uncover. As molecular sequencing technologies have advanced, the microbial world has been increasingly opened up to investigation by biologists. It is now widely accepted that microorganisms have always played—and still continue to play—many important roles in the lives of plants and animals (McFall-Ngai et al. 2013, Douglas and Werren 2016). Symbiotic interactions between microbes and macrobes have been documented among diverse organisms, and many researchers maintain that all macrobes engage in symbiotic interactions with microbes in natural settings (Zilber-Rosenberg and Rosenberg 2008, Bosch and McFall-Ngai 2011, Dupré and O'Malley 2009, Gordon et al. 2013, Singh et al. 2013, Booth 2014).

The term “holobiont” was coined by Lynn Margulis and used to refer to symbiotic associations that last throughout a significant portion of an organism’s lifetime (Margulis 1991). The term first found wide usage in coral biology where it was defined as a coral colony and its associated photosynthetic algal symbionts and bacterial communities (Rohwer et al. 2002, Knowlton and Rohwer 2003, Stat et al. 2012). The recent influx of interest in macrobe-microbe relations has led to a proliferation of the term “holobiont”, now most often understood as a host macroorganism and *all* of its associated microbiota, including bacteria, archaea, viruses, protists, fungi, and microscopic multicellular animals such as nematodes (Zilber-Rosenberg and Rosenberg 2008, Booth 2014, Bordenstein and Theis 2015, Moran and Sloan 2015, Douglas and Werren 2016, Theis et al. 2016). Because the holobiont includes *all* associated microbiota, the interactions between holobiont partners may be harmful, beneficial or of no consequence. Relationships between partners may be coevolved or opportunistic, competitive or cooperative.

Recognition that holobionts are common in nature has led many researchers to reassess their views about various processes and concepts that are foundational in biological thinking. Dupré and O'Malley (2009) was an important early step in this direction, suggesting that microbial biology has radical implications for the future development of many areas of philosophy of biology. Philosophers are not alone here. They are joined by biologists in calling for a transformation in our thinking. “Right now, for those of us who are not evolutionary biologists, it is enough to recognize that the very foundations of biology are being shaken by both the integration of microbiology into concepts of macroevolution and the recognition that host-microbe symbioses are a major theme in biological systems” (McFall-Ngai 2016). One suggestion is that there is now a need to “upgrade fundamental theories” because holobiont systems “raise the discussion of individuality and organismality beyond its historical perspective to a level that challenges and extends current thinking” (Bordenstein and Theis 2015).

Criteria for individuating entities are of central importance in biology (Hull 1978, 1980, 1992; Buss 1987; Godfrey-Smith 2009, 2013; Clark 2011; Pradeu 2012). For example, population biologists and ecologists must be able to distinguish individuals in a population. Evolutionary biologists must be able to distinguish parents from their offspring, and one lineage from another. Immunologists and physiologists must be able to distinguish between an individual and its environment. Organisms have long been the paradigm of individuality—a horse, a tree, a human—both within and outside of biology (Aristotle 1984). Extensive experimentation and theoretical

advances in biology, especially within the last century, have changed our understanding of how individuals can and did evolve (Buss 1987, Maynard Smith and Szathmary 1995, Michod 1999). New conceptions of individuality have helped us to understand individuality across the biological hierarchy: genes, cells, multicellular organisms, superorganismal colonies, and multi-species symbiotic communities (Dawkins 1976, Sober and Wilson 1989, Queller and Strassmann 2009).

The question motivating this paper is: are holobionts biological individuals or communities? I start by introducing the biology of the coral holobiont as an example of the complexity and diversity of the interactions within a holobiont. I take the coral holobiont to be a good example of typical holobiont dynamics, and as such an appropriate test case for working through whether or not holobionts are biological individuals. In the following sections I expand on relevant accounts of biological individuality and claims made in favor of holobionts being biological individuals. I then consider whether holobionts meet some plausible and common criteria for either evolutionary individuality (reproduction and heritability) or organismality (functionally integrated interactors, metabolic collaboration, or cooperative low-conflict consortiums). I conclude that most holobionts share more affinities with communities than they do with individual wholes, and that, except for in rare and possibly unrealized cases, holobionts *do not* meet the criteria for being evolutionary individuals, units of selection, or organisms.

The Coral Holobiont

The evolutionary and ecological success of corals in the characteristically nutrient-poor environments of tropical and subtropical oceans is thought to be a direct consequence of their ability to form mutually beneficial symbioses with unicellular photoautotrophic dinoflagellates in the genus *Symbiodinium*, commonly referred to as zooxanthellae (Stat et al. 2012, Lesser et al. 2013). The appearance of coral reefs in the Triassic has been attributed to the evolution of the symbiotic association between the coral host and *Symbiodinium*.

Corals are mainly colonized by free-living *Symbiodinium* from the environment, but a direct transfer of *Symbiodinium* from parent to offspring via inclusion in the egg also occurs in many brooding corals, and is occasionally seen in some spawning corals (Thompson et al. 2015). The *Symbiodinium* are endosymbionts that reside within the cells of the coral host. They fix carbon through photosynthesis, which they provide, along with other nutrients, to their host in return for host waste metabolites and protection from grazing (Stat et al. 2012, Roth 2014). This exchange of nutrients is mutually beneficial and helps the coral secrete the calcium carbonate skeletal structure that is shared by the coral colony and contributes to the formation of coral reefs. This endosymbiotic association is especially interesting because it involves two eukaryotic organisms and the genome of the symbiont is three times larger than the genome of its host (Roth 2014).

The total number of bacterial species associated with corals remain largely unavailable, but recent estimates put the number between 3000-6000 species (Stat et al. 2012). At least some of the coral-associated prokaryotes are beneficial to the coral host. Cyanobacteria provide nutrition through nitrogen fixation (Lesser et al., 2004), and bacteria residing in the coral's exterior mucous layer act as a first line of defense against pathogens by producing antimicrobial compounds and occupying space (Stat et al. 2012). Most coral-associated fungi and viruses are thought to be parasitic (Golubic et al. 2005), but there may be exceptions. There is evidence that some endolithic fungi residing in the coral skeleton could be converting nitrate and nitrite to ammonia which could enable fixed nitrogen to cycle within the coral holobiont, and some of the

bacteriophages might be helping to beneficially regulate associated bacterial communities (Wegley et al. 2007). With rare exceptions, the microorganismal component of the coral holobiont is obtained from the environment (Apprill et al. 2009).

There is a substantial amount of genetic diversity within *Symbiodinium* and evidence that some of it is reflected as functional diversity. This is important because corals can harbor more than one type of *Symbiodinium* at a time, and are known to shuffle and switch out their *Symbiodinium* types. The coral holobiont is very sensitive to changes in ocean temperature and lives close to their upper thermal tolerance limit. A prolonged temperature increase of as little as 1 °C causes stress and can lead to coral bleaching, the forceful expulsion of the *Symbiodinium* by the coral host. The coral holobiont is a dynamic system with members fluctuating on a daily basis depending on the environmental conditions and life-cycle requirements (Thompson et al. 2015).

For example, corals associated with clade C *Symbiodinium* usually perform better than corals with clade D *Symbiodinium* in normal conditions with clade C-infected juveniles growing two to three times as fast as those infected with clade D (Lesser et al. 2013, Hume et al. 2016). But in hot environments where coral bleaching is common, corals with Clade D *Symbiodinium* do better. One reason is that clade D appears to be more heat-tolerant than Clade C, and may rapidly increase the heat stress tolerance of corals (Lesser et al. 2013, Thompson et al. 2015, Hume et al. 2016). This can lead to short-term benefits during periods of thermal stress, but corals dominated by clade D *Symbiodinium* show significantly decreased growth and reproduction in the long term. This suggests that the *Symbiodinium* switch between being mutualists and parasites of the host depending on current environmental conditions.

It has also been suggested that things are not what they seem for the *Symbiodinium* either. The coral host might be benefiting at the expense of their microbes by capturing and controlling its algal symbionts (Garcia and Gerardo 2014). Wooldridge (2010) suggests that “the coral host exerts a ‘controlled parasitism’ over its algal symbionts that is akin to an enforced domestication arrangement” with the “...the coral host as an active ‘farmer’ of the energy-rich photoassimilates from its captive symbionts.”

Which lineages come together to make up a coral holobiont is strongly contingent on environmental conditions and cooperation and conflict between different possible partners. This flexibility in lineage composition allows the coral to respond to the abiotic environment in ways that it wouldn't be able to if it was on its own, and was likely important for the success of corals. Understanding the mechanisms and relations that have evolved between the coral and its symbiotic partners is crucial for understanding coral biology.

Controversy about the Status of Holobionts

Several biologists and philosophers have claimed that holobionts, or similar multi-lineage assemblages of macrobes and microbes, constitute at least one level of organization at which natural selection acts. Biologists Zilber-Rosenberg and Rosenberg were the first to articulate what they call the “hologenome theory of evolution,” which they see as an alternative to “currently accepted dogma,” according to which the units of selection are individual organisms as traditionally conceived (2008, 731). The hologenome is a collective unit made up of all of the host and microbial genomes of the holobiont. They write, “In the hologenome theory of evolution, we suggest that the holobiont... with its hologenome, acting in consortium, should be

considered a unit of selection in evolution...” (2008, 723). They are not the only theorists to have made such claims:

- “Therefore the holobiont, i.e. the host including all symbionts, should be regarded as the unit of selection as the association between host and symbionts may affect the fitness of the holobiont depending on the environment” (Feldhaar 2011).
- “The hologenome theory of evolution considers the dynamic holobiont as a single unit for natural selection and provides a more accommodating view of evolution blending Darwinism and Lamarckism” (Singh et al. 2013)
- “[A]n organism’s genetics and fitness are inclusive of its microbiome” (Brucker and Bordenstein 2014).
- “Thus, the holobiont, with its integrated community of species, becomes a unit of natural selection whose evolutionary mechanisms are largely unexplored” (Gilbert 2014).
- “The hologenome concept is a holistic view of genetics in which animals and plants are polygenomic entities. Thus, variation in the hologenome can lead to variation in phenotypes upon which natural selection or genetic drift can operate” (Bordenstein and Theis 2015).

Without referring to holobionts explicitly, philosophers Dupré and O’Malley¹ endorse a similar view about the fundamental entities that are operated on by natural selection: “...complex systems involving the collaboration of many highly diverse lineage forming entities. This sort of interactor, we also suggest, is the most fundamental unit of selection.” (Dupré and O’Malley 2009). Ereshefsky and Pedroso (2013, 2015) use biofilms as a case study to defend the position that multi-species consortiums can be units of selection.

Other authors have been critical of the claim that holobionts are important units of selection. Moran and Sloan (2015) state that “While biologists would agree that microorganisms have important roles in host evolution, this statement is a far cry from the claim that they are fused with hosts to form the primary units of selection, or that hosts and microorganisms provide different portions of a unified genome” and that “...some observations that superficially appear to support the concept of the hologenome have spawned confusion about real biological issues.” Douglas and Werren (2016) state that “...it is highly unlikely that the entire microbiome will evolve as a “holobiont” with its host” and that “...the hologenome concept is unhelpful to the study of host interactions with resident microorganisms...”

Pushing back against recent criticism, Theis et al. (2016) have backed off a bit from claims that holobionts are always units of selection, but still consider the holobiont to be a level at which selection acts. They also reiterate that shifts in the microbial community are akin to changes in allele frequency in the host genome, suggesting that the hologenome is a single unit upon which selection acts.

¹ O’Malley has since moved away from this view, recently stating that natural selection probably does not act at the collective level in multilineal systems, of which holobionts are one kind (O’Malley 2016).

Biological Individuality

I will start with some general remarks about biological individuality, before moving on to considerations about whether or not holobionts are biological individuals. Accounts of biological individuality tend to be clustered around a few different investigatory projects. These accounts are not exclusive, a particular biological entity may be an individual of more than one type.

Genealogical individuals are lineages such as species and phylogenetic taxa (Hull 1978). They are the units that can evolve. Evolutionary individuals are the individuals of natural selection (Sober and Wilson 1998, Ereshefsky and Pedroso 2015, Clarke 2016). They are the units upon which natural selection operates and members of a population that has the capacity to evolve.

The traditional target of accounts of biological individuality is the organism, the phenomenologically discrete living entities inhabiting the world around us. I contrast organisms with evolutionary individuals in that the defining criteria of organismal individuality are not restricted to purely evolutionary considerations. As a first pass, organisms are bounded individuals that are functionally or metabolically integrated. They are systems with mutually dependent components that work together to maintain the system's structure or developmental trajectory (e.g., Pradeu 2010, Godfrey-Smith 2013). There are numerous accounts of organismality, many of which don't agree².

One other approach to organismality is one that focuses physiological individuality. This is a family of views that have developed somewhat independently of evolutionary views about individuality. Of these, the immunological account advocated by Pradeu (2012) is especially promising. On this account, the boundaries of physiological individuals (organisms) are established by the immune system of the host. Other nearby physiological views rest on the fact that symbionts are either critical for host development, or make something of the host work, that is, realize or help realize an important physiological function (See, for example, Bocci 1992, Berg 1996, Xu and Gordon 2003)³. This line of thinking has been important for understanding the boundaries, health and development of macroorganisms, especially large and complex vertebrates like us.

I will not discuss the relation between holobionts and physiological individuality for two reasons. First, I am concerned with starting from a more general analysis that doesn't privilege macroorganisms or index claims of individuality to the host. I take physiological individuality to be host-centric, subordinating microbe individuality to functional, immunological, and developmental considerations regarding the host. My focus is: how might higher level individuality emerge out of the general interactions between macrobes and microbes? Second, much of the controversy and debate surrounding holobionts has focused on whether holobionts ought to be understood as units of selection or communities, and the present paper is an attempt to take side in that controversy. Because of these reasons I will limit myself to evolutionary individuality and accounts of organismality that focus on functional integration of a collaborative or codependent nature.

Are Holobionts Evolutionary Individuals?

² See Clarke (2011) for a thorough survey of accounts of organismality and individuality.

³ I thank Thomas Pradeu for emphasizing this point, as well as pointing out the gap in my treatment of physiological individuality in a previous draft.

Evolutionary individuals are entities defined in terms of natural selection: they vary among each other, their variability causes variations in fitness, and that variation and fitness effect is heritable (Lewontin 1970, Godfrey-Smith 2009, Ereshefsky and Pedroso 2015, Clarke 2016). The Darwinian population framework is one way to make evolutionary individuality more precise. This framework has its roots in the account of natural selection articulated in Lewontin (1970), and gets its name from Godfrey-Smith's (2009) extended update of that account. Building from Lewontin's three criteria of *variation*, *heredity* and *differences in reproductive success*, a *Darwinian population* is defined as “a collection of causally connected individual things in which there is variation in character, which leads to differences in reproductive output (differences in how much or how quickly individuals reproduce), and which is inherited to some extent” (2009, 39). A member of such a population is a *Darwinian individual* (Godfrey-Smith 2009, 40). Darwinian individuals are units of selection, and as such are “the loci of causal action for the process of selection” (Booth 2014, 664). If holobionts are to be evolutionary individuals on an account like this, then they must (a) be able to reproduce at the level of the holobiont, and (b) there must be heritable differences at the level of the holobiont. I will argue in the next section that holobionts fulfill neither of these criteria.

Holobiont Reproduction

Godfrey-Smith (2015) makes further distinctions that are helpful for understanding the generation of new entities. He starts with the notion of recurring structures, which may either be reproducing or reconstructed. Reproducing things form parent-offspring lineages, whereas reconstructed ones do not. Reconstructed objects include organs and enzymes. Reproduction can be collective, simple, or scaffolded (Godfrey-Smith 2009). Collective reproducers include multicellular organisms. Simple reproducers, such as bacteria, can give rise to more things like themselves. Scaffolded reproducers rely on external machinery for their reproduction. Examples include genes and viruses.

An alternative analysis of reproduction, Griesemer's (2000, 2014, 2016) “reproducer” account, shares many similarities with the Darwinian individual account. It differs in that he emphasizes the necessity of material overlap between generations. “Reproducers are entities with the capacity to multiply because offspring bear relations of ‘material overlap’ with their parents” (Griesemer 2016). What makes reproduction different than recurrence or mere production is the conveyance of developmental cycles linked together in a lineage.

Griesemer and Godfrey-Smith agree that reproduction requires the formation of lineages. Their disagreement centers on the necessity of material transfer between generations and how to parse the divisions between scaffolded and collective reproduces (Griesemer 2016). For Griesemer, nearly all organisms are scaffolded in some way because they depend on some aspect of the environment for either their development or reproduction.

Questions about whether holobionts are evolutionary individuals—i.e., natural selection operates at the level of the holobiont—are intimately related to questions about holobiont recurrence. Specifically, is the generation of a new holobiont the result of reproduction or reconstruction, which is to ask: do holobionts reproduce as a whole such that there holobiont-level lineages? The answer depends primarily on how the microbial symbionts are transmitted.

The transmission routes by which microbial symbionts move from host to host vary considerably and are usually divided into two categories (Bright and Bulgherisi 2010). Vertical transmission is

the direct transfer of symbionts from the host parent(s) to their offspring. Horizontally transmitted symbionts are acquired from other non-parental hosts or from free-living population in the environment. The majority of microbial associations of multicellular animals and plants are thought to be horizontally transmitted (Moran and Sloan 2015). Cases of vertical transmission are often not obligate, that is, even though the symbionts can be, or even often are, transmitted vertically, they can also be obtained horizontally. Obligate vertical transmission is thought to be rare, and may reliably occur only in cases of endosymbiosis. Many cases of vertical transmission are really part of a mixed-mode of transmission. Though it appears to be the case that rates of vertical transmission are exceedingly low relative to the rates of horizontal transmission, it is perhaps the case those microbes that are transmitted vertically play a relatively larger role in the lives of their hosts⁴.

Few, if any, holobionts as individual units are reproducers because strict vertical inheritance is rare. If the set of lineages that make up the holobiont varies within and between host generations, then the holobiont cannot be a coherent unit of selection. More select partnerships between hosts and individual symbionts do in rare cases meet the criteria for reproduction, such as eukaryotes and their mitochondria or corals that pass along *Symbiodinium* in their eggs. High partner fidelity is fleeting without strict vertical transmission. If the component lineages can all go their separate ways between reproductive events and reassemble at a later time in at least a semi-random fashion, then there are no higher-level lineage connecting generations of holobionts.

The only way to draw something approximating parent-offspring relations between holobionts without vertical transmission of *all* the component lineages is to privilege one of the partners. This is nearly always the host, as it is the largest and likely the longest living part of a holobiont. Privileging the host is nearly inevitable, as holobionts are defined by picking out particular hosts with all of their associated microbes.

But what if we privileged one of the other collaborating lineages? Consider the human + gut-microbiota holobiont with a different emphasis. A bacterium lives inside a doctor who hasn't been particularly careful about sanitation. The doctor goes into work and delivers an unrelated baby. The gut bacterium replicates and one of the offspring bacteria quickly colonizes the infant. A new holobiont is assembled in the collaboration of the human lineage and doctor's bacterial lineage. We can now pick out a new parent-offspring relation between the doctor holobiont and the baby holobiont. From a lineage-neutral perspective at the holobiont level, this is no stranger than saying that the parent-offspring relation is between the mother holobiont and the baby holobiont.

More sensibly, we can say that there is no fact of the matter about what constitutes the parent-offspring relation between host-microbe associations without vertical transmission. Perhaps even better: there are no parent-offspring relations between holobionts in these cases. The related concepts of parenthood and reproduction have simply been stretched too far, and most holobionts are marginal reproducers at best (Godfrey-Smith 2009, 2011, 2013; Booth 2014). In cases of horizontal transmission, the host-microbe associations recur in each generation, but they do not reproduce as a unit and do not form lineages (Godfrey-Smith 2012, Booth 2014). A particular host-microbe association might even be obligatory for the reproduction of one the partners. But the reproductive events wouldn't be holobiont reproduction, rather they would be co-dependent

⁴ I thank Thomas Pradeu for emphasizing this point.

scaffolded reproduction of partner lineages. That is to say, they are not evolutionary individuals on this account.

Holobiont Heritability and Holobiont Lineages

Holobiont reproduction with vertical transmission in itself is not sufficient for evolutionary individuality. Horizontal transmission or symbiont exchange during the host's lifetime can disrupt heritability. Even hypothetical holobionts where all of the microbial symbionts are vertically transmitted during host reproduction may not be evolutionary individuals. The second important consideration regarding selection at the holobiont level is whether or not there is partner fidelity: a stable association of host and symbionts across multiple host generations. High partner fidelity is a prerequisite for evolutionary individuality because the holobiont can only evolve as a unit if the host and its symbionts co-occur across multiple host generations. Only holobionts with both a high degree of vertical transmission and high partner fidelity will meet the criteria for evolutionary individuality.

Partner fidelity is expected to be highest when there is obligate vertical transmission, though high partner fidelity might also be possible in holobiont systems with horizontal transmission where the hosts provide their offspring with symbionts and where specificity is high (Douglas and Werren 2016). Partner fidelity is often imposed by vertical transmission because the microbial partners will have a strong selective interest in the reproductive fitness of the host when their fitness is tied to the reproductive success of the host. But partner fidelity and vertical transmission can come apart. Partner fidelity is expected to be lower when there are no obligate dependencies, or when the obligate dependencies can be supplied by many different symbiont partners. If partner lineages can jump ship, and horizontally transfer to other hosts, then the different parts of a holobiont aren't locked into a common fate. This leads to an expectation of increased conflict between the members of the holobiont as they "pursue their own goals"; namely, selection for increased replication of one's own lineage at the expense of the success of the multi-lineage holobiont. As conflicts of interests among partners increase (e.g., due to weak partner fidelity), then the holobiont is undermined as a higher-level unit of selection.

This is especially apparent if we consider a hypothetical coral holobiont where all partners have long life-spans. For example, coral A and dinoflagellate symbiont C are distinct lineages that interact to form coral holobiont α . Coral B and dinoflagellate symbiont D are distinct lineages that interact to form coral holobiont β . If the fates of those lineages are tied to the success of the holobiont that they help produce, then we have some notion of alignment of fitness. But if the collaborating lineages are independent of each other, it is possible that lineages C and D could switch partners. In that case A and D would interact to form a new holobiont, γ , and B and C would interact to form the new holobiont δ . Holobionts γ and δ survive, and lineages A, B, C and D eventually reproduce and make it into the next generation of holobionts.

The extinction of coral holobionts α and β did not prevent the proliferation of the lineages that produced them. The death of α and β might have fitness consequences for the individual lineages, but it needn't. In this example, the overall success of the holobiont(s) will have fitness consequences for the individual lineages that make them up, but the dissolution of any particular holobiont—because of either partner death or partner switching—needn't necessarily have fitness consequences for the individual lineages. Holobionts γ and δ could reproduce as whole units and still wouldn't be units of selection without high partner fidelity over the life of the

partner lineages. A high degree of symbiont swapping will undermine selection at the level of the holobiont because horizontal swapping continually dissolves and creates individual holobionts over the course of the lifetimes of the individual partner lifetimes.

Yet again, it is difficult to pick out what counts as a case of a new or different holobiont without explicitly privileging one of the partners. I take this as a likely reason for why the concept of the holobiont rests on macrobe bias. Microbes are small. Macrobes are big. Microbes go where their microbial associates go. Holobionts *seem* contiguous to us, in ways that symbiotic associations between macrobe-macrobe symbiotic associations like plants and pollinators don't. But this alone does not indicate that they are part of some larger whole. It is just an artifact of their size.

Indexing holobiont identity to the host is not without its benefits. Focusing on the larger and longer lived host makes it easier to demarcate holobionts, a necessity for tracking holobiont changes over time and in response to environmental changes. Indexing symbiont community identity to the host is appropriate when the host is of primary interest⁵. But in the context of evolutionary individuality, all partners have equal weight, regardless of their size or longevity. Evolutionary individuals are only picked out by being entities that natural selection works on, not any physical or taxonomic features. Mistaking holobionts for units of selection appears to stem, in part, from host-centric thinking, macrobe bias and a reification of operational concepts like hologenome, microbiome, and metagenome⁶.

Are Holobionts Organisms?

So far I have only discussed accounts of biological individuality that are tied to reproduction at the level of the holobiont. Another approach that has been proposed is based on David Hull's interactor account of individuality (Hull 1980, 1992), itself based on Richard Dawkins' replicator theory (Dawkins 1976). According to Hull, an interactor is "an entity that directly interacts as a cohesive whole with its environment in such a way that replication is differential" (Hull 1980). The replicators are entities which "pass on their structures largely intact from generation to generation" (Hull 1980). Replicators were originally conceived as an abstraction of the role of gene, while interactors are an abstraction of the role of organisms. The interactor is often identified as the primary unit of selection, but this role has also been extended to the replicator (Lloyd 2012). The replicator-interactor framework has since been put to powerful use in the analysis of the complexities of inheritance and interaction in symbiotic consortiums (Sterelny 2001, 2004, 2011).

Criticism of the replicator-interactor framework has primarily focused on problems with replicator transmission and the reproduction of interactors (see for example, Griesemer 2000, Godfrey-Smith 2009). This has led some to suggest a notion of biological individuality that maintains interactors while decoupling them from replicators or particular reproductive requirements (O'Malley and Dupré 2009; Dupré 2012; Ereshefsky and Pedroso 2013, 2015). For example, Dupré (2012) makes the following claim: "...the organisms that are parts of evolutionary lineages are not the same things as the organisms that interact functionally with their biological and non-biological surroundings." This is immediately followed by a much

⁵ See (Sterelny 2006) for a similar argument regarding the individuation of ecosystems.

⁶ See Huss (2014) for an extended discussion and warning about reifying categories such as metagenome, microbiome and enterotype.

stronger claim: “The latter, which I take to be more fundamental, are composed of a variety of the former, which are the more traditionally conceived organisms” (Dupré 2012).

The move to an interactor-only, or “updated interactor”, concept of individuality is meant to capture the fact that many multilineage symbiotic consortiums appear to function as organisms in their environments, while also recognizing that the many replicators coming together within these consortiums are not inextricably tied together. It is suggested that all that is needed for an entity to be an interactor is enough interaction between the member parts such that the success or failure of the interactor has a unitary effect on the success or failure of its members (Ereshefsky and Pedroso 2013). For example, a higher survivorship in the members than if the members were living independently from each other (Ereshefsky and Pedroso 2013).

In the case of holobionts, much more needs to be said about the necessary type and strength of interactions between holobiont partners such that the holobiont interacts as a functional whole (organisms) with the environment⁷. I will examine two approaches to organismality that may be compatible with the view that holobionts are modified interactors and biological individuals. The first is an approach that focuses on functional integration through metabolic collaboration. The second is the cooperation and conflict framework outlined in Queller and Strassmann (2009).

Functional Integration and Metabolic Dependency

Metabolic dependencies are a hallmark of the close symbiotic relationships that inspired the adoption of the holobiont framework. A type of biological individuality that emerges from lineage-forming entities collaborating in metabolism is a position explored by Dupré (2012) and Dupré and O’Malley (2009).

“My colleague Maureen O’Malley and I (Dupré and O’Malley 2009) have suggested that the most fundamental way to think of living things is as the intersection of lineages and metabolism. The point we are making is that, contrary to the assumption that is fundamental to the one genome, one organism idea, the biological entities that form reproducing and evolving lineages are not the same as the entities that function as wholes in wider biological contexts. Functional biological wholes, the entities that we primarily think of as organisms, are in fact cooperating assemblies of a wide variety of lineage-forming entities.” (Dupré 2012)

In their view of the natural world, “collaboration” among entities of fundamentally different types is essential to all living systems (Dupré and O’Malley 2009)⁸. Collaboration encompasses cooperation and competition, includes metabolic, structural, and fitness-affecting interactions, and involves entities at many levels of biological organization. Dupré and O’Malley do not discuss holobionts, and so it is unclear whether they would endorse the position that holobionts are organisms, but their collaboration criteria for organismality seems a promising option for those wishing to argue that multilineage systems like holobionts are biological individuals.

⁷ One reviewer suggested that many of the claims about holobionts as units of selection found in the quotations presented in the section entitled “Controversy about the Status of Holobionts” be interpreted as claims about holobionts being interactors. I disagree that this is the correct interpretation of the presented views. At the very least, it is unclear exactly what the quoted authors mean when they say holobionts are a unit of selection.

⁸ Bouchard (2009) presents a similar view, arguing that “superindividuality” can emerge in persistent, functionally-integrated, multispecies communities.

Individual holobionts will almost inevitably contain partnerships that vary across the full range of collaboration as described by Dupré and O'Malley (2009). There are at least two reasons to be cautious about such a permissive approach to collaboration, if that collaboration is to be the glue that binds lower level individuals into a higher level individual.

First, recurring interactions, even ones with reciprocal benefits, needn't indicate that there is functional integration or active collaboration. Members of a particular host species will inevitably share similar physiologies, microbial defense mechanisms, and biochemistries compared to other species. The fact that selective microbial communities with the same composition are always associated with the same hosts may be due to differences in community assembly rather than cooperative behavior or a shared evolutionary history. Similar communities are to be expected across common similar environments.

Second, the evolution of metabolic dependencies or codependencies among host-microbe communities does not mean that the community, in this case a holobiont, is functionally integrated into a whole. When a nutrient is routinely provided by an organism's environment, selection for biochemical efficiency can lead to loss of genes in the particular biochemical pathway (Morris et al. 2012). The Black Queen Hypothesis states that this process is able to occur whenever there are microbial communities where "leaky products" are produced by different members that are routinely associated with each other (Morris et al. 2012; Douglas and Werren 2016). Such processes can lead to interdependent communities without requiring selection or functional integration at the holobiont level (Sachs and Hollowell 2012; Douglas and Werren 2016). Mushegian and Ebert (2016) give plausible examples that include protective symbioses based on secondary metabolic functions, such as detoxification of heavy metals or plant toxins, or production of defensive compounds against other microbes, which are likely to be beneficial regardless of whether the microbe is in a host or non-host environment. Perhaps a more compelling example of the independence of metabolic dependency and functional integration is the mammalian gut. Mammalian digestive tracks provide microorganisms with all sorts of beneficial "leaky products" such as warmth, moisture and nutrients. Commensalist bacteria utilize and may even depend on those resources, while the host is entirely unaffected by the bacterial presence, by definition.

Is recurrence with metabolic integration enough to infer that holobionts are whole entities in their own right rather than mere associations of individuals or ephemeral communities? No, because ecological communities can have reproducible dynamics and predictable outcomes for their members without being the result of selection, integration or coevolution at the level of the community (Mushegian and Ebert 2016). Something further is needed to bind individuals into a whole. As argued in earlier sections, reproduction and cotransmission at the level of the whole are plausible options. Another possibility is cooperation.

Cooperation and Conflict

The second conception of organismality I will consider is the framework developed in Queller and Strassmann (2009, see also Queller and Strassmann, this special issue⁹), which takes a social behavior approach to defining organisms. This approach is probably the most amendable to treating multi-species symbiotic consortiums like holobionts as organisms. Queller and Strassmann (2009) define organisms as "the largest unit of near-unanimous design." They fill

⁹ Queller and Strassman (this issue) argue that it is extremely unlikely that any holobionts qualify as organisms.

this out by saying that “the organism is simply a unit with high cooperation and very low conflict among its parts. That is, the organism has adaptations and it is not much disrupted by adaptations at lower levels” (Queller and Strassmann 2009).

Conflicts of interest between the symbiont partners is a major obstacle to holobiont individuality. Within a single species, conflicts of interest can be suppressed by maintaining genetic homogeneity in the case of an individual multicellular organism, or high genetic relatedness in the case of a group of cooperating organisms. These routes are not available to multi-species holobionts.

Three factors that are important for conflict suppression in symbioses are: vertical transmission, specificity of symbiont relationships, and little or no dependency on a free-living state (Herre et al. 1999; Sachs et al. 2011, Lesser et al. 2013). Vertical transmission favors increased metabolic integration and can lead to symbiont genome reduction and obligate dependencies between partners, which in turn lowers conflict because the symbionts share a common fate (Sachs et al. 2011, Lesser et al. 2013). A high specificity in symbiont relationships reduces the number of competitive phenotypes that a host has to contend with, and reduces the conflict between individual microbes associated with the host. Little or no dependency on a free-living state reduces the chance that a symbiont will have to contend with selective pressures from two environments, leading to specialized adaptation to symbiotic environments, and increasing the likelihood of coevolution and dependencies between partners.

These three factors can lead to the symbionts sharing common interests and an alignment of fitness. The difference between having mutually beneficial relationships and sharing common interests is a key difference between being just a group of interacting individuals and being a higher level individual. The open question is: how often does this happen?

We see again that there is a problem when we look at the holobiont as a whole unit. Some of the host-microbe relations might meet criteria for multi-species organismality, but it seems highly unlikely that *all* of the host-microbe relations will be cooperative. Even if there is cooperation without conflict between a host and all of its microbial symbionts, there will still inevitably be conflict in the holobiont between the microorganisms. For example, microbes will compete for resources within the host, including nutrients and space, as well as for access to the next host generation. The host will remain the site of a whole ecosystem of complex microorganismal interactions (see Mushegian and Ebert 2016 for a similar view). The idea that there is a single interaction between the host and its microbiome is an artifact of macrobe bias and the difficulty of gathering information about all the various host-microbe interactions. Epistemic limitations shouldn't tempt us to overly simplistic conclusions about biological individuality.

The Disunity of the Holobiont

It is unlikely that there is any holobiont that is also an evolutionary individual or organism if the holobiont is defined as a macrobe host and *all* of its associated microorganisms. It is not impossible that a host and its symbionts could form a unit of selection, it is just that the conditions are unlikely to obtain. High partner fidelity and alignment of fitness are necessary. This is achieved by vertical inheritance or by strong mutual partner choice. Such high-fidelity associations are unlikely to occur across all of the partnerships within a holobiont. Where it does not, selective pressures at the level of the individual lineages will tend to put the partners into direct competition or active exploitation. Focusing on the processes, interactions, and relations

that occur between holobiont partners like this opens up a suite of questions. Does vertical transmission lead to increased metabolic integration and alignment of fitness? Or are these necessary before vertical transmission becomes permanent? To what degree are holobiont partnerships species-specific coevolved consortiums vs. generalist assemblages taking advantage of leaky products or stable environments? What is the relationship between different biological parameters: mode of transmission vs. alignment of fitness vs. metabolic integration?

Many of these questions concern ecological relationships. As such, holobiont theory and research will be impoverished if it doesn't incorporate the powerful theoretical tools of community and ecosystem ecology. As we saw with the coral holobiont, holobionts are complex systems comprised of an array of lineages interacting in diverse ways. Holobionts are disunified in the sense that they share features of both individuals and communities. Some partner interactions are best considered as symbioses—ranging from mutualism to parasitism—where the partners mutually form a part of each other's environments. Other interactions long ago bound the individual lineages together into a higher-level lineage and evolutionary individual. I expect there are plenty of indeterminable cases on the road between ecological interaction and becoming a full-fledged individual. Holobionts are interesting because they share features of organisms and communities. Neither reducing the holobiont to a set of pairwise interactions between symbiont partners nor treating the entire community as a single biological individual is a universally appropriate approach.

References

- Apprill A, Marlow HQ, Martindale MQ, Rappe MS (2009) The onset of microbial associations in the coral *Pocillopora meandrina*. *ISME J* 3:685–699
- Aristotle (1984) *Categories*. In: Barnes J (ed) *The complete works of Aristotle*, vol. 1. Princeton: Princeton University Press.
- Berg RD (1996) The indigenous gastrointestinal microflora. *Trends Microbiol* 4:430–435
- Bocci V (1992) The neglected organ: bacterial flora has a crucial immunostimulatory role. *Perspect Biol Med* 35(2):251-60
- Booth A (2014) Symbiosis, selection and individuality. *Biol Philos* 29:657-673
- Bordenstein SR, Theis KR (2015) Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol* 13(8):e1002226
- Bosch TCG, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. *Zoology* 114:185-190
- Bouchard F (2013) What is a symbiotic superindividual and how do you measure its fitness? In Bouchard F and Huneman P (eds) *From groups to individuals: evolution and emerging individuality*. MIT Press, Cambridge, pp 243-264
- Bright M, Bulgheresi S (2010) A complex journey: transmission of microbial symbionts. *Nat Rev Microbiol* 8(3):218-230
- Brucker RM, Bordenstein SR (2014) Response to comment on “The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*.” *Science* 345:1011
- Buss L (1987) *The evolution of individuality*. Princeton: Princeton University Press

- Clarke E (2011) The problem of biological individuality. *Biological Theory* 5(4):312-325
- Clarke E (2016) Levels of selection in biofilms: multispecies biofilms are *not* evolutionary individuals. *Biol Philos* 31:191-212
- Dawkins R (1976) *The Selfish Gene*. Best Books
- Douglas AE (2008) Conflict, cheats and the persistence of symbioses. *New Phytol* 177:849–58
- Douglas AE, Werren JH (2016) Holes in the hologenome: Why host-microbe symbioses are not holobionts. *mBio* 7(2):e02099-15
- Dupré J (2012) The polygenomic organism. In: Dupré J (ed) *Processes of life: essays in the philosophy of biology*. Oxford University Press, Oxford, pp 116–127
- Dupré J, O'Malley MA (2009) Varieties of living things: life at the intersection of lineage and metabolism. *Philosophy and Theory in Biology*, 1
- Ereshefsky M, Pedroso M (2013) Biological individuality: the case of biofilms. *Biol Philos* 28(2):331–349
- Ereshefsky M, Pedroso M (2015) Rethinking evolutionary individuality. *PNAS* 112: 10126-32
- Feldhaar, H (2011) Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology* 36:533-543
- Garcia JR, Gerardo NM (2014) The symbiont side of symbiosis: do microbes really benefit? *Frontiers in Microbiology*. 5(510):1-6
- Gilbert SF, Sapp J, Tauber AI (2012) A symbiotic view of life: we have never been individuals. *Quar Rev Biol* 87(4):325-341
- Gilbert SF (2014) Symbiosis as the way of eukaryotic life: the dependent co-origination of the body. *J Biosci* 39:201–209
- Godfrey-Smith P (2009) *Darwinian populations and natural selection*. Oxford University Press, Oxford
- Godfrey-Smith P (2011) Agents and acacias: replies to Dennett, Sterelny, and Queller. *Biol Philos*
- Godfrey-Smith P (2013) Darwinian Individuals. In: Bouchard F, Huneman P (eds) *From groups to individuals: evolution and emerging individuality*. MIT Press, Cambridge, pp 17–36
- Godfrey-Smith P (2015) Reproduction, symbiosis, and the eukaryotic cell. *PNAS* 112(33):10120-10125
- Golubic S, Radtke G, Le Campion-Alsumard T (2005) Endolithic fungi in marine ecosystems. *Trends Microbiol* 13:229–235
- Gordon J, Knowlton N, Relman DA, Rohwer F, and Youle M (2013) Superorganisms and holobionts. *Microbe* 8(4):152-153

- Griesemer J (2000) Development, culture and the units of inheritance. *Philosophy of Science* 67:S348–S368.
- Griesemer J (2014) Reproduction and scaffolded developmental processes: an integrated evolutionary perspective. In: Minelli A, Pradeu T (eds) *Towards a Theory of Development*. New York: Oxford University Press. pp 183-202
- Griesemer J (2016) Reproduction in complex life cycles: toward a developmental reaction norms perspective. *Philosophy of Science*. online first
- Herre EA, Knowlton N, Mueller UG, Rehner SA (1999) The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol Evol* 14:49–53
- Hull D (1978) A matter of individuality. *Philosophy of Science* 45(3):335-360
- Hull D (1980) Individuality and selection. *Annu Rev of Ecol Syst* 11:311-332
- Hull D (1992) Individual. In: Fox-Keller E and Lloyd E *Keywords in evolutionary biology*. Cambridge, Mass: Harvard University Press. pp 181-187
- Hume BCC, Voolstra CR, Arif C, D'Angelo C, Burt JA, Eyal G, Loya Y, Wiedenmann J (2016) Ancestral genetic diversity associated with the rapid spread of stress-tolerant coral symbionts in response to Holocene climate change. *PNAS* 113(16):4416-4421
- Huss J (2014) Methodology and ontology in microbiome research. *Biol Theory* 9:392-400
- Knowlton N and Rohwer F (2003) Multispecies microbial mutualisms on coral reefs: The host as a habitat. *The American Naturalist* 162:S51-S62
- Lesser MP, Stat M, Gates RD (2013) The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. *Coral Reefs* 32:603-611
- Lewontin RC (1970) The units of selection. *Annu Rev Ecol Syst* 1(1):1–18
- Lloyd E (2012) Units and levels of selection. In: Zalta EN (eds) *The Stanford encyclopedia of philosophy*. Winter 2012. <http://plato.stanford.edu/archives/win2012/entries/selection-units/>
- Margulis L (1991) Symbiogenesis and symbiogenesis. In: Margulis L, Fester R (ed), *Symbiosis as a source of evolutionary innovation: speciation and morphogenesis*. MIT Press, Cambridge, MA. pp 1-14
- Maynard Smith J, Szathmary E (1995) *The major transitions in evolution*. Oxford University Press, Oxford, United Kingdom.
- McFall-Ngai M (2016) Introduction to the hologenome special series. *mBio* 7(2):e00371-16
- McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci. U.S.A.* 110:3229–3236
- Michod RE (1999) *Darwinian dynamics. Evolutionary transitions in fitness and individuality*. Princeton: Princeton University Press.

- Moran NA (2006) Symbiosis. *Curr Biol* 16(October):R866–R871
- Moran NA, Sloan DB (2015) The hologenome concept: helpful or hollow? *PLoS Biol* 13(12):e1002311
- Morris JJ, Lenski RE, Zinser ER (2012) The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. *mBio* 3(2):e00036-12
- Mushegian AA, Ebert D (2016) Rethinking “mutualism” in diverse host-symbiont communities. *Bioessays* 38:100-108
- O’Malley MA (2016, in press) Reproduction expanded: multigenerational and multilineal units of evolution. *Philosophy of Science*
- Pradeu T (2010) What is an organism? An immunological answer. *Hist Philos Life Sci* 32(2–3):247–267
- Pradeu T (2012) *The limits of the self: immunology and biological identity*. Oxford University Press, USA, Translated by Elizabeth Vitanza
- Queller DC, Strassmann JE (2009) Beyond society: the evolution of organismality. *Phil Trans R Soc B* 364:3143-3155
- Queller DC, Strassmann JE (this issue) Problems of multi-species organisms: endosymbionts to holobionts. *Biol Philos*
- Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* 243:1-10
- Rosenberg E, Koren O, Reshef L, Efrony R and Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* 5:355–362
- Roth MS (2014) The engine of the reef: photobiology of the coral-algal symbiosis. *Frontiers in Microbiology*. 5(422):1-22.
- Sachs JL, Essenberg CJ, Turcotte MM (2011) New paradigms for the evolution of beneficial infections. *Trends Ecol Evol* 26:202–209
- Sachs JL, Hollowell AC. 2012. The origins of cooperative bacterial communities. *mBio* 3(3):e00099-12
- Singh Y, Ahmad J, Musarrat J, Ehtesham NZ, Hasnain SE (2013) Emerging importance of holobionts in evolution and in probiotics. *Gut Pathog* 5:12.
- Sober E, Wilson DS (1998) *Unto others: the evolution and psychology of unselfish behaviour*. Harvard University Press, USA
- Stat M, Baker AC, Bourne DG, Correa AMS, Forsman Z, Huggett MJ, Pochon X, Skillings D, Toonen RJ, van Oppen MJH, Gates RD (2012) Molecular Delineation of Species in the Coral Holobiont. In: Lesser M, (ed) *Advances in Marine Biology*, Vol. 63. Academic Press, The Netherlands: Amsterdam, pp 1-65.

- Sterelny K (2001) Niche construction, developmental systems, and the extended replicator. In: Oyama S, Griffiths PE, Gray RD (eds) *Cycles of contingency: developmental systems and evolution*. Cambridge, Mass: MIT Press. pp 333-349
- Sterelny K (2004) Symbiosis, evolvability and modularity. In: Schlosser G, Wagner G (eds) *Modularity in Development and Evolution*. University of Chicago Press.
- Sterelny K (2006) Local ecological communities. *Philosophy of Science*. 73:215-231
- Sterelny K (2011) Darwinian spaces: Peter Godfrey-Smith on selection and evolution. *Biol Philos* 26:489–500
- Theis KR, Dhelly NM, Klassen JL, Brucker RM, Baines JF, Bosch TCG, Cryan JF, Gilbert SF, Goodnight CJ, Lloyd EA, Sapp J, Vandenkoornhuysse P, Zilber-Rosenberg I, Rosenberg E, Bordenstein SR (2016) Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. *mSystems* 1(2):e00028-16.
- Thompson JR, Rivera HE, Closek CJ, Medina M (2015) Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. *Frontiers in Cellular and Infection Microbiology* 4(176):1-20. doi: 10.3389/fcimb.2014.00176
- Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F (2007) Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. *Environmental Microbiology* 9(11):2707-2719
- Wooldridge SA (2010) Is the coral-algae symbiosis really ‘mutually beneficial’ for the partners? *Bioessays* 32:615–625
- Xu J, Gordon JI (2003) Honor thy symbionts. *Proc Natl Acad Sci USA* 100:10452–10459
- Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol Rev* 32:723–735