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Problems of multi-species organisms: endosymbionts to holobionts

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Abstract

The organism is one of the fundamental concepts of biology and has been at the center of many discussions about biological individuality, yet what exactly it is can be confusing. The definition that we find generally useful is that an organism is a unit in which all the subunits have evolved to be highly cooperative, with very little conflict. We focus on how often organisms evolve from two or more formerly independent organisms. Two canonical transitions of this type—replicators clustered in cells and endosymbiotic organelles within host cells—demonstrate the reality of this kind of evolutionary transition and suggest conditions that can favor it. These conditions include co-transmission of the partners across generations and rules that strongly regulate and limit conflict, such as a fair meiosis. Recently, much attention has been given to associations of animals with microbes involved in their nutrition. These range from tight endosymbiotic associations like those between aphids and *Buchnera* bacteria, to the complex communities in animal intestines. Here, starting with a reflection about identity through time (which we call "Theseus's fish"), we consider the distinctions between these kinds of animal–bacteria interactions and describe the criteria by which a few can be considered jointly organismal but most cannot.

Keywords

Organismality
Individuality
Major evolutionary transitions
Mutualism
Cooperation

Theseus's fish

The marine isopod *Cymothoa exigua* has a very exotic lifestyle. It takes up residence in the mouth of a fish, snappers or pompanos, for example~~,~~ ostensibly serving as a replacement tongue for the fish (Brusca and Gilligan 1983). The isopod itself creates the need for a replacement tongue by damaging the original, causing it to atrophy to a small stump to which the crustacean attaches itself with perepod hooks. The isopod fits snugly within the fish's buccal cavity, feeding, growing, and reproducing, while the host fish also seems to feed normally (Parker and Booth 2013).

The initial question we pose here is how replacement of one organ in this way affects the status of the organism. Is it still the same organism, a new kind of combined organism, or just an organism with another organism in its mouth? To pose the question in an even starker way, imagine that it is not just the fish's tongue that is replaced, but all its body parts. Perhaps another isopod species somehow replaces its eyes, an annelid worm serves as its intestine, and so on. We will call this fanciful being Theseus's fish, after the Greek mythological character, Theseus who had a ship that had all of its parts replaced over the decades. It is a common view that Theseus's ship retains its identity through these replacements, with one argument being that it continues to function in the same shiply way. This argument still carries some force even if some of the replacement parts were repurposed from other technologies—the sail from a tent or the rudder from a plow. Even if we decide it is not the same ship, we can shift the question a bit and ask if it is still a ship at all. This is the question we want to ask about Theseus's fish. Is it still an organism at all? Does it still function like an organism?

The question may seem ridiculous, or at least contrary to the biological convention that requires that individual organisms be members of a single species. But biology provides us with real examples (Table 1). The mitochondrion is, figuratively speaking, the isopod tongue of eukaryotic cells. It replaces or augments the energetic pathways of its host cell and yet it is derived from a different species, indeed from a different kingdom (Archibald 2015). One of the questions of this essay is how often and how this kind of merging happens. Theseus's fish may be problematic as an organism but it cannot be dismissed simply because it is formed of more than one species. Although Theseus's fish is fanciful, it is a useful thought experiment relevant to various claims that multispecies assemblages are organismal in nature (Table 2). The extreme for such assemblages may occur when a large number of microbe species are associated with a eukaryote, such as an animal with an internal digestive system housing millions of diverse bacteria. How organismal is such an assemblage and how does it differ from the relationship between mitochondrion and host, or isopod and fish? The idea that the holobiome is an organism itself, ~~as~~or the unit of adaptation, has been claimed by some (Bordenstein and Theis 2015; Bosch and Miller 2016; Gordon et al. 2010; Rosenberg and Zilber-Rosenberg 2013; Zilber-Rosenberg and Rosenberg 2008), though such claims have been critiqued (Douglas and Werren 2016; Hester et al. 2016; Moran and Sloan 2015). Below we assess what it would take for both simple and complex species assemblages to be considered organismal.

Table 1

Organismal egalitarian interactions (two fraternal) from this paper

Entity 1	Entity 2	Vertical trans-mission?	Cooperation; what 2 does for 1 (see text)	Conflict below controlled?	Selecte referen
Allele A	Allele B at same locus	No	Various physiological functions	Yes; fair meiosis; veil of ignorance	Brandv and Co (2015) Lindho al. (20 Queller Strassn (2013)
Eukaryote cell	Mitochondri~~a,~~on or chloroplast	Yes, through female	Energy pathways	Single parent transmission	Archib (2015) Gray (1993) Burt an Trivers (2006) Chase (2007)
Braconid wasp	Polydnavirus	Yes	Virulence to caterpillar host	Yes	Pichon (2015) Strand Burke (2014)
Aphid	*Buchnera*	Yes	Essential amino acids	Egg bottleneck for *Buchnera*	Dale a Moran (2006) Werneg and Mc (2001) McCut and Mc (2012)
Cell 1	Cell 2, multicellularity fraternal	~~Yes~~Kin selected	Yes	Single cell bottleneck	Grosbe and Strathn (2007)

Entity 1	Entity 2	Vertical transmission?	Cooperation; what 2 does for 1 (see text)	Conflict below controlled?	Selecte referen
Honeybee queen	Workers, fraternal	~~Yes~~Kin selected	Yes, defense, foraging	Yes, through worker policing	Seeley (1989)

Table 2

Two or more species interactions that are not organismal

Entity 1	Entity 2	Vertical transmission?	Cooperation; what 2 does for 1 (see text)	Conflict below controlled?	Selected referenc
Fish	Isopod, *Cymothoa exigua*	No	Temporary shared feeding; tongue function	No	Brusca a Gilligan (1983), Parker a Booth (2013)
Insect	*Wolbachia*	Sometimes	Various	Not well	Werren e al. (2008 Ahmed e al. (2015
Beewolf wasp	*Streptomyces*	Yes	Fungicide	Yes	Kaltenpo et al. (2010)
Termite	*Bacteria*, protists	Some	Digestion	Some	Mikaely et al. (2015), et al. (2015)
Plant	Pollinator	No	Pollination	No	
Acacia tree	Ant	No	Protection	Yes	Bronstei (1998), Palmer a Brody (2013)

Entity 1	**Entity 2**	**Vertical trans-mission?**	**Cooperation; what 2 does for 1 (see text)**	**Conflict below controlled?**	**Selected referenc**
Legume	Rhizobia bacteria	No	Nitrogen	Yes, plant carbon restriction	Kiers et (2003)
Water buffalo	Oxpecker	No	Ectoparasite removal	No; also pick at flesh	Plantan al. (2013 Weeks (2000)
Host ant	Slave worker	No	Bring in food; defend	Some slaves fooled; slave rebellion	Pamming et al. (2012), Czechow and Godzińsk (2015)
Cuckoo	Host bird	No	Care for chick	Some fooled some not	Davies (2010), Langmor et al. (2005)
Angler fish	Prey	No	Fish eats prey	No	Pietsch a Grobeck (1978)
Zebrafish	Gut microbes	No	Digest food	No	Burns et (2016)
Coral	Bacteria	No	Metabolism	No	Hester et (2016)
Holobiont; animal	Gut microbes	Very rare	Digestion	No	Gordon al. (2010 Turnbau et al. (2007), Ley et al (2008)

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Organismality

The ancient idea that organisms seem functional or purposeful was given an explanation by Darwin’s theory of evolution. Organisms have heritable variation and those variants that confer higher fitness—whether by augmenting food intake, avoiding predators, having more babies, or any other means—increase in the population so individuals come to have traits that appear to have been designed for these purposes, all subsidiary to the ultimate function of elevating fitness.

To apply a functional approach to the isopod-tongued fish we need to address what its function or functions might be. In the explanation given above, function applies most directly to the mutational variant; its effects determine whether the trait increases or not. But we more often see function as also applying to the organism as a whole. The reason is that organisms are units of consolidated function or adaptation (Queller 1997; Queller and Strassmann 2009; Strassmann and Queller 2010). When one allele benefits its own reproduction, it also benefits the reproduction of its organism, or more accurately, an allele gains its own advantage *through* increasing the reproduction of the organism in which it resides.

We have found it useful to go beyond recognizing the functional and purposeful nature of organisms and to actually *define* organisms using these characteristics. The organism is a biological unit with high cooperation and very low conflict among its parts so that “the organism has adaptations and it is not much disrupted by adaptations at lower levels” (Queller and Strassmann 2009). Another way to put it is that the constituents of an organism have many mutually beneficial interactions and very few that are harmful. They are very extreme mutualists.

This perspective situates our definition of organisms within the traditions in social evolution, multi-level selection, and the major evolutionary transitions (Buss 1987; Hull 1978; Maynard Smith and Szathmáry 1995; Michod 1999). Selection can occur at various levels in the hierarchy of life and the locus of individuality or organismality can change from one to another. We prefer “organismality” to “individuality” because of our emphasis on functional adaptive coherence; for example species have been argued to be individuals (Hull 1978; Okasha 2006), but they are never organisms. Likewise, the idea of a Darwinian individual (Godfrey-Smith 2013) is developed around what kinds of units have the properties required for selection to operate, where ours reflects the adaptive result of selection. Although our definition emerges from the units-of-selection debate, it is in itself atheoretical. The organism is defined by its empirical functional characteristics, which can then be explained by selection theory.

Most of the various definitions of “organism” invoke integration and function, with physiological or biochemical pathways that causally interact and work together (Pepper and Herron 2008; Pradeu 2010; Santelices 1999; Strassmann and Queller 2010). Our definition emphasizes the “work together” part; interaction alone is not enough. Few would consider the malarial parasite *Plasmodium* to be part of the same organism as its host, even though their physiologies interact causally in manifold ways. The reason is that their physiologies are not integrated towards a common function. Instead there is conflict; each is adapted to thwarting many of its partner’s functions.

While we think that our organism concept successfully captures all organisms that are agreed upon by biologists, it does opens the door to some less conventional ones. An organism could in principle fit our definition if it were spatially dispersed, or composed of transiently interacting parts, or built up of different species (Queller and Strassmann 2009). It might turn out that these characteristics are absent or rare in entities that show organismal functionality, but if we want to understand how organismality evolves, we should not rule these factors out a priori but instead study the issue empirically.

New levels of organismality have evolved in the history of life (Maynard Smith and Szathmáry 1995). From a primordial soup, groups of primitive replicators were collected into cells. Cells, in turn, sometimes combine into higher-level organisms, either through merging different species as in the eukaryotic cell with its mitochondrial and chloroplast organelles, or through conventional multicellularity (Grosberg and Strathmann 2007). Arguably, multicellular individuals can also combine into organisms (often called superorganisms) as in the more derived large-colony social insects (Seeley 1989).

In this paper we will not attempt to argue or referee the varying definitions of organismality or individuality, most of which, like ours, rely strongly on functionality and purpose (Pepper and Herron 2008; Santelices 1999). Our goal is to explore whether Theseus's fish and real examples of multi-species aggregates can be considered to be consolidated units of function, with very high cooperation and very low conflict among their parts. We will use the word "organism" for those properties but our arguments do not hinge on using this word.

The organism is not restricted to one particular level in the hierarchy of life. Organismality sometimes steps up a level, as when multicellular organisms evolve from unicellular ancestors or when organismal social insect colonies evolve from solitary ancestors (Maynard Smith and Szathmáry 1995). The new higher-level entity can be considered organismal once it evolves extensive cooperation and very reduced conflict among its subunits. There are two kinds of these transitions in organismality, which we call fraternal and egalitarian, after the social two-thirds of the French revolutionary slogan (with libertarians being the non-social third class) (Queller 1997). The examples mentioned above, multicellular organisms and social insect colonies, are fraternal associations are among kin (Queller 1997). Their cooperation can be favored via kin selection and some parties can be selected to give up reproduction entirely in order to promote the spread of their genes in relatives (workers in social insects, somatic cells in multicellular bodies).

The advantage of indirect fitness from helping relatives is closed off to cooperation among different species, which are our concern in this paper. These are egalitarian associations, egalitarian not in the sense of strict equality but in the more limited sense that each party must continue to have some reproduction if it is to be an evolutionary contributor to the cooperative association. Egalitarian associations—mutualistic partnerships that benefit both partners—are very common in nature (Bronstein 2015; Douglas 2010). However, although they emerge from mutualisms, egalitarian transitions to organismality are much rarer. More attention has been given to the fraternal major transitions (Queller 2000) but in this paper we focus on egalitarian ones.

Would Theseus's fish constitute an egalitarian organism? We should ask whether the parts interact in a manner that is essentially completely cooperative and free of actual conflict. This is an empirical question for which we would need to demand more information on the hypothetical Theseus's fish, but we can answer it for the *Cymothoa* isopod and its fish host. First, the isopod-tongued fish (or fish-bodied isopod) seems an unlikely candidate for an organism it does not fulfill conditions that are thought to favor the evolution of egalitarian cooperation among non-relatives (conditions discussed in more detail later). One such condition is co-transmission of reproductive propagules; when all parties reproduce through the same propagules, they should all be selected to enhance this joint reproduction or vertical transmission (Estrela et al. 2016). The isopod and its fish host do not have shared propagules or jointly coordinated reproduction, so one can easily suppose that the isopod might be selected to reproduce more than is good for the fish, while the fish is selected to reproduce more than is good for the isopod.

Another condition favoring egalitarian cooperation is a lifelong partnership that favors partner fidelity feedback such that cooperative investments in the partner can eventually yield returns to the investor (Bull and Rice 1991). Many fish never acquire the isopod and those that do may lose them as they get larger (Parker and Booth 2013), so one can envision the isopod being selected to try to extract all it can from the fish host before the partnership ends. Of course, the real partnership might confound these theoretical expectations, but it appears not to. In aquaculture studies numerous deleterious effects of the isopods on their hosts have been noted, including tissue damage, anemia, and inhibited growth, with the last also being documented in wild populations (Parker and Booth 2013). One species of the isopod was called the snapper-choking isopod (Joca et al. 2015) and some of the harmful effects may be due to reduced respiration by either restricting flow of water over the gills or by directly damaging the fish's gills ~~by beating their own respiratory structures~~ (Parker and Booth 2013). This kind of competitive reproduction by component parts is likely to be generally important in ruling out multi-species groups as organisms.

If we could ask the fish whether it liked its isopod, it would surely reply (unless its isopod tongue was firmly in cheek) that it did not. It seems quite clear that the isopod-tongued fish is not a single cooperative organism but rather a host organism with a harmful parasite organism. In fairness to the authors of the original tongue replacement hypothesis (Brusca and Gilligan 1983), their claim was more limited—that once the fish's original tongue was gone, it was better to have the isopod there than not to have it. But it does serve as a colorful example of how to pursue the organism question for multi-species associations. If simply replacing a tongue is fraught with difficulty, it is no surprise that Theseus's fish has never evolved.

Still, the examples of the mitochondrion and the chloroplast (which are described in detail later in this paper) show that organisms can form from different species, so it is worth examining the question for other mutualistic associations. It is possible that egalitarian transitions in organismality are more common than we think (Queller and Strassmann 2009). Acquiring a symbiont that has already perfected certain functions on its own can be by far the most rapid way of acquiring novel functionality, providing conflicts can be controlled (Oliver et al. 2010). These would not have to be major transitions in the sense of being a big event in the history of life like the acquisition of mitochondria. They might be limited and local but nevertheless novel entities that are fully cooperative. However, we should hold them to roughly the same standards of high cooperation and low conflict shown by generally accepted organisms. If it turns out that egalitarian transitions truly are rare, then we would also like to understand why that is so.

Selection for aiding another species

There has been considerable thinking on what factors would favor helping an individual of another species (Bronstein 2015; Herre et al. 1999; Leigh 2010; Sachs et al. 2004). First, there must be the ability to perform beneficial acts. Some such benefits are simply incidental by-products of actions that are beneficial to the actor itself. Pollinators gain by acquiring nectar or pollen for food but only incidentally carry pollen to fertilize other plants. Things become more interesting when individuals are selected to help their partners because improving the condition or number of the partners means that they will produce more of the desired by-products, not because they are repaying a favor, but because that is what they do (Connor 1986). For example, ants that protect their acacia tree host get a healthier, larger host that will have more nesting sites (hollow thorns) and produce more leaflets, each of which bears a Beltian body that the ants alone can harvest as food (Bronstein 1998; Orona-Tamayo et al. 2013; Palmer and Brody 2013). Complementarily, an acacia that provides more of these benefits will have more ants to protect it. These mutual investments can lead to a virtuous circle of benefits, although conflicts usually remain.

These cycles work only if the benefits return to the investors (or its relatives) instead of to someone else, so partner fidelity is an important factor selecting for mutualisms (Bull and Rice 1991; Foster and Wenseleers 2006; Sachs et al. 2004). When partners reproduce through the same propagules, something we call co-transmission, fidelity is extended into future generations and becomes even more powerful. It means that current investments could even be returned to your offspring. Co-transmission is not a part of all mutualisms but it is an important element of the accepted egalitarian major transitions, as we will show below. But co-transmission is rarely perfect, which means there remains some opportunity for one partner to gain at the expense of the other.

For a partnership to become organismal, there need to be mechanisms that regulate or control conflict of this kind. These are sometimes called policing mechanisms but the mechanisms may be simpler than that word implies. For example, individual resources could be blocked off and privatized (Strassmann and Queller 2014); in a legume-rhizobium relationship, the former initially possesses the carbon compounds and the latter the nitrogen (Kiers et al. 2003). Each partner might succeed at getting more of its missing resource than its partner is selected to give, but it is also possible that initial possession allows control and that robbery is not achievable. Without such controls, non-organismal conflict would persist.

Lessons from grouping different genes in cells

Arguably the first egalitarian transition was when different replicators in the primordial soup formed the first primordial groups. This transition is far enough in the past that we cannot say much about it with real confidence. But members of groups containing replicators with beneficial and complementary effects would tend to produce more replicator offspring. Selfish reproduction is limited by enclosure within a cell that produces daughter cells as opposed to, say, lysing and allowing the replicators to independently disperse and reassemble. Viruses are replicators ~~that~~ can indeed use the latter strategy, showing how harmful this kind of reproduction can be to the host. A virus that replicates this way should not be considered part of the organism. However, it could evolve to become part of the host organism if it becomes integrated into the host DNA, performs some useful function, and replicates via normal host channels. Polydnaviruses do just this~~,~~. ~~particularly~~For example ~~in~~certain parasitoid wasps of the braconidae and ichneumonidae ~~where they~~ use the viruses to ~~transfer virulence genes~~attack their hosts and virus replication machinery is completely integrated in the wasps (Pichon et al. 2015;

Strand and Burke 2014).

Strict co-transmission is also subverted by sexual reproduction as well as by bacterial genetic exchange mechanisms. Consider a sexual diploid individual resulting from the fusion of two haploid gametes. The different genes are only partly co-transmitted and do not have the same fitness. We are able to pretend that they do because of our focus on the individual, which can have only one fitness. But any particular allele in that individual will end up in approximately half of the individual's offspring and it is expected to share only half of this fitness with an allele at any other unlinked locus. In principle, each could be selected to increase its own fitness at the expense of the individual's overall fitness. So how is it that these entities with different fitnesses have evolved to cooperate to such a remarkable degree instead of pursuing their own unique fitnesses? The remainder of the answer must come from policing, where the members of the collective have the power to suppress selfish behavior of other members (Beekman and Ratnieks 2003; Ratnieks and Visscher 1989). In eukaryotes a collection of genes encodes the rules of meiosis that keep most alleles from preferentially getting into offspring (Brandvain and Coop 2015). Equality is also enforced by a veil of ignorance (Queller and Strassmann 2013), with genes generally being unable to preferentially identify and aid those offspring that bear copies of the same gene.

Meiotic drive genes occasionally find ways to get around the rules of meiosis and gain a selfish advantage (Lindholm et al. 2016). Similarly, selfish genetic elements such as transposons can gain through their own horizontal reproduction pathways. When this is harmful to the other genes, some of these genes may evolve to suppress the selfish element but a small amount of conflict can remain even in the most canonical of organisms (Foster 2011). They are units of *near*-unanimous cooperation but are rarely in perfect accord.

Four lessons about organismality emerge from this discussion of gene collectives. First, co-transmission of these genes is usually high and this helps account for their organismal cooperation in cells. Second, co-transmission is not usually sufficient for organismal cooperation; there also need to be mechanisms that reduce conflict. Third, both devices are imperfect and some conflict can remain even within the most organismal units. Finally, the union of unrelated sets of genes in every generation of a sexual species drives home the point that organisms can emerge from unrelated sub-units, making it worthwhile to search for other such organisms.

Intracellular endosymbionts

Endosymbionts live inside the bodies of their hosts, often within host cells. Many have lost the ability to live independently. One of the canonical types of egalitarian major transitions originated in intracellular endosymbiosis. The modern eukaryotic cell includes one or more types of internal organelles derived from endosymbiosis, with the mitochondrion originating from an alpha-proteobacterium and the chloroplast from a cyanobacterium (Gray 1993). The intracellular lifestyle seems ideal for promoting an egalitarian merger when there is complete co-transmission to the next generation, with the host and symbiont reproducing asexually to make two new cells.

However, sexual reproduction by the host brings potential problems (Burt and Trivers 2006; Partridge and Hurst 1998). When two gametes fuse, the merging of the two endosymbiont populations could lead to fierce competition. The common solution to this problem is for only one parent to transmit the symbiont, with symbionts from the other parent being killed. This solution, however, causes another problem because now co-transmission is incomplete. When symbionts are passed only through the female line, as is usually true for mitochondria and chloroplasts, symbionts may be selected to harm the host’s male reproduction if that generates any increase in female reproduction. For example, there are many mitochondrial mutants that cause male sterility in a wide variety of hermaphroditic plants and there are also many host autosomal genes that evolve to restore male fertility (Chase 2007). Hosts have several advantages that may usually keep selfish symbionts under control. Their genomes are larger than those of the symbionts probably giving them a mutational advantage in evolutionary conflicts. The symbiont genomes are often further disadvantaged because they are subject to considerable drift and degradation owing to small population sizes and asexual reproduction (McCutcheon and Moran 2012; Wernegreen 2002). Finally, if a host can successfully disguise its male versus female functions, the mitochondria will not be able to take separate actions.

Have endosymbionts other than mitochondria and chloroplasts been involved in organismal transitions? Intracellular endosymbionts are surprisingly widespread in many environments, based on DNA amplification using specific 16~~s~~S primers. They are often beneficial to their host but can also be harmful (Wernegreen 2012). Only the beneficial ones are candidates for organismality in our sense; the harmful ones fail the condition of very low conflict. But the bar is much higher than that; they must be highly cooperative and show very little conflict, to a similar degree as mitochondria and chloroplasts in the eukaryotic cells.

Many of the best-studied endosymbionts are in insects. Insects with highly specific diets have endosymbionts that help digest the food or provide specific amino acids and vitamins that are missing (Dale and Moran 2006). For example, some aphids are supplied with certain amino acids and vitamins by *Buchnera* bacteria housed in specialized cells called bacteriocytes (Moran and Telang 1998).

Buchnera is highly evolved in concert with its host. These bacteria are passed down vertically from mothers to offspring and colonize the bacteriocytes early in development (Wernegreen and Moran 2001). This co-transmission helps reduce conflict, as is the case for mitochondria and chloroplasts. The egg constitutes a bottleneck that keeps the symbiont population small and genetically uniform, which selects for cooperation among the symbionts. Again, co-transmission is through eggs only, which creates potential conflict over production of males, but no such conflict has been reported in *Buchnera*. And again, the power of the symbionts to engage in conflict is reduced as a consequence of this small population size; extreme genome reduction and degradation means that fewer genes can be brought to bear in conflicts and selection is weaker on them (McCutcheon and Moran 2012). The degree of cooperation is such that neither party can survive without the other and little or no conflict is known. We therefore consider *Buchnera* and other intracellular endosymbionts with similar properties to be the same organism as their host, for the same reasons that mitochondria and chloroplasts are the same organism as their hosts.

Symbionts need not be intracellular and co-transmission need not be through eggs. The European beewolf is a wasp that harbors specialized *Streptomyces* bacteria in its antennae, which it uses to apply the symbiont to its brood cells. The larvae then take up the symbiont, which provides protection against fungi as the larvae develop in their damp soil chambers (Kaltenpoth et al. 2010). This is still co-transmission, which might be conducive to evolving organismality.

But even intracellular endosymbionts are not always organismal. *Wolbachia* bacteria colonize many arthropod species and are passed on largely vertically through the host eggs. Although they have some mutualistic effects, they also have a variety of detrimental effects on the host (Werren et al. 2008; Zug and Hammerstein 2015). For example, *Wolbachia* can transform isopod male hosts, which cannot transmit *Wolbachia*, into females, which do (Rigaud et al. 1997). Such detrimental effects may evolve because *Wolbachia* has retained more power in conflicts with the host relative to other intracellular endosymbionts. Though transmission is usually vertical, non-trivial horizontal transmission is suggested both by occasional host switching and by *Wolbachia's* retention of transposable elements, which are usually lost in non-recombining ancient endosymbionts (Ahmed et al. 2015; Moran and Plague 2004). There is also genetic evidence for recombination (Werren et al. 2008). This ability to recombine may make *Wolbachia* more evolutionarily potent than many other intracellular endosymbionts.

Fellow travellers versus hitchhikers

Are there additional egalitarian organisms beyond the canonical ones and a few additional endosymbiotic alliances? We are open to the possibility (Queller and Strassmann 2009) but the bar has been set high. Cooperation does not suffice; there must be very high cooperation and very low conflict, just as in standard organisms. Our organismal endosymbionts are fellow travellers, not mere hitchhikers. They are fellow travellers both in the literal sense of being partly co-transmitted but also in the figurative sense applied to communist sympathizers who are not members of the party but buy into the party line and make common cause with it. This squares nicely with our congress/party metaphor for organisms made up of different parties, like host cell and mitochondrion, each being a set of genes in an organism with common interests, but with partly divergent interests with other parties (Strassmann and Queller 2010).

Hitchhikers may travel together to some degree but have less intense cooperation and more conflict. The oxpecker is an African bird that can be seen riding on the backs of ungulates like antelopes and water buffalos. It performs the task of picking ticks and other arthropods of off their hides (Mooring and Mundy 1996; Nunn et al. 2011). Though it is difficult to demonstrate, this is likely a non-trivial benefit when the arthropods can be vectors of disease. But however large the benefit, no one would confuse the oxpeckers as part of their water buffalo host. For one thing, they flit from one host to another and they spend much of their time elsewhere, at a roost or at their nest. Although we have not made fidelity an explicit part of our organism definition, it is one of the factors that promotes high cooperation. More to the point of our definition, oxpeckers do not seem to be extensively cooperative with their host and there is conflict. They make a living from their hosts but are not dedicated to them. Indeed, when the opportunity arises to gain at the expense of the host, they will do so, as when they pick at a host's open wounds (Plantan et al. 2013; Weeks 2000), not a trivial harm when wounds can become infected. Such conflicts often occur within mutualistic interactions (Douglas 2008; Herre et al. 1999), and only the ones where these conflicts are reduced to very low levels should be considered organismal.

Willing workers versus slaves and dupes

Our assertion that an organism is an entity with near-perfect cooperation among its parts needs a modest amendment to exclude certain partnerships that no one would regard as organismal. By cooperation, we mean not just the appearance of behavioral cooperation but adaptive beneficial cooperation. Sometimes an association will appear to be behaviorally cooperative but this cooperation is not adaptive or beneficial for one partner. For example, so-called slave ants are captured when they are pupae by another species and then grow up to become behaviorally cooperative workers in the captor colony, which functions quite harmoniously, though slave rebellions can occur (Pamminger et al. 2012). Similarly, a parent bird victimized by a cuckoo seems to willingly invest enormous energy to raise the cuckoo chick as its own. But both cuckoo and slave ant have been evolutionarily duped by partners exploiting their cooperative tendencies evolved for other reasons (Davies 2010). Host genes have never evolved to benefit their cuckoo chicks, nor slave ants to benefit their captors. Indeed, each tends to evolve to escape these partnerships if they can (Czechowski and Godzińska 2015; Langmore et al. 2005). If we were to include these partnerships as organismal, would we not also have to include the "partnership" of an angler fish and its prey that is attracted to the lure dangling from the anglerfish's forehead (Pietsch and Grobecker 1978)? The prey appears to act cooperatively, swimming toward the lure and the anglerfish's mouth. But it has clearly not evolved to benefit the anglerfish—it has been duped. So the amendment necessary to our definition is that each party is not just cooperative, but must have evolved to benefit its partners. Likewise, it must have evolved extremely little harm to partners.

Are complex holobionts organismal?

Holobiont is an old term in the symbiosis literature for the entity made up of symbiotic partners (Meyer-Abich 1950). Lynn Margulis formally defined the term: “holobiont: symbiont compound of recognizable bionts” (Margulis 1991). More recently the holobiont term has usually been applied to a eukaryote host with the whole community of microbes associated with it—protists, bacteria, Archaea, viruses, as well as unicellular fungi and algae (Gordon et al. 2010) and this is the sense that we will use. However, as we have already discussed simple endosymbiotic holobionts, here we will focus especially on the more complex end of the range. Specifically, we are interested in holobionts composed of the host with a highly diverse gut microbiome, which have been the subject of much study and speculation (Turnbaugh et al. 2007).

As a noun referring to a specific association, the term "holobiont" is useful, but care must be taken with respect to additional implications about how holobionts arise, how stable they are, how functionally integrated they are, or how natural selection operates on them. Some argue that a holobiont is similar to a superorganism, a sort of organism made up of organisms, which has its own division of labor, specialized reproductives, and sophisticated communication networks (Bordenstein and Theis 2015; Bosch and Miller 2016; Gordon et al. 2010; Rosenberg and Zilber-Rosenberg 2013). Furthermore it has become associated with the idea that the holobiont has a combined “hologenome”, such that microbes can be viewed as analogous to the nuclear genes of an organism (Bordenstein and Theis 2015; Bosch and Miller 2016; Zilber-Rosenberg and Rosenberg 2008). Such views have been criticized previously (Douglas and Werren 2016; Hester et al. 2016; Leggat et al. 2007; Moran and Sloan 2015) and here we critique them in the specific context of organismality.

Perhaps the best way to consider whether a holobiont is an organism is to examine another well-established complex case, the social insects. Wheeler (1911) proposed that social insect colonies be called superorganisms because he viewed them as organismal entities made up of other organisms. We have argued that social insect colonies can be superorganisms that fit our definition of an organism. Indeed, all organisms are really superorganisms in the sense that they have evolved from previously independent units (Queller and Strassmann 2009). Ant, bee, or wasp colonies generally consist of highly related individuals with largely coincident interests. Selection at the level of the colony is not particularly controversial here since the individuals that make up colonies share both genes and reproductive fates. This kind of social organism evolved from maximally related groups of a single once-mated female with daughters that remain as helpers to rear the subsequent brood (Hughes et al. 2008). Social insects may retain potential conflict over issues such as who produces the males, or the sex ratio of the queen's offspring (Queller and Strassmann 1998). In some social insects such conflicts can be costly (Chapuisat et al. 1997) to a degree that we would not consider their colonies to be organismal. But in some species, such as the honey bee *Apis melifera*, such conflicts appear to be very minimal, such that their colonies would seem to qualify as organisms (Seeley 1989).

If the social insect colony can be viewed as an organism, can the same be said of a holobiont? Does it also show the required unanimity of interest and suppression of conflict? Certainly there are often beneficial effects flowing from microbes to host and vice versa with hosts sometimes suffering when their microbiome is disturbed (Khosravi and Mazmanian 2013). But most benefits to the host could be simply byproducts of microbial metabolism—important, but not evolved to benefit the host. The question that needs to be answered by future research is exactly how much cooperation and conflict there is within holobionts. In the meantime, we can ask if complex holobionts possess the kinds of traits that appear to be conducive to evolving egalitarian organismality. In general, they do not.

Most complex holobionts, such as a human and its gut microbiome, lack long-term fidelity. Many holobionts can be somewhat loosely associated with hosts. Their component members can change rapidly, for example with dietary shifts (David et al. 2014). Even when some component bionts are dependent on others for certain metabolic products, the specific other can be fungible. Moreover, many invertebrate gut microbial species spend considerable time outside the host (Ley et al. 2008) so any cooperative traits they do have are potentially subject to adaptive tradeoffs due to selection in alternative environments. They are unlikely to be fully committed to a host when they also have to take care of business elsewhere. Finally, gut microbiota are not, for the most part, transmitted vertically from parent to offspring (Palmer et al. 2007) and so they may each be selected to favor their own reproductive options over those of the other holobiont members. There are interesting exceptions where vertical transmission is more common, though by no means universal, as in termites, which acquire some of their symbionts by eating the feces of colonymates, but the diversity of microbes in the termite gut still makes extensive conflict-free cooperation unlikely and unstable (Mikaelyan et al. 2015; Tai et al. 2015).

The large number of individuals and species of microbes in a complex holobiont compounds the problem of cooperation. Each faces the potential temptation to defect, perhaps shirking on its cooperative output in order to reproduce a bit more. Cost-free byproduct benefits are of course still possible, but perhaps not the costly investments in others. Unlike endosymbionts, where reproductive bottlenecks promote genetic uniformity and the potential for cheating, the human gut microbiome has many species with a huge collective amount of genetic variation. For these kinds of reasons, no ecologist would assert that a community of macroorganisms, perhaps a forest, is organismal, even if there are some beneficial interactions. Any such claim for a community of microorganisms needs to be treated very skeptically.

For understanding how complex holobionts arise and are maintained, a more appropriate framework than the superorganism would probably come from community ecology. Community ecology studies the forest, not the trees; it is particularly important for understanding holobionts because it deals with complex and variable species interactions and with how communities are assembled and how they change over time. The study of zebrafish gut microbial communities and their development illustrates the approach of community assembly (Burns et al. 2016). The authors begin with a null model of ecology: that microbial communities in zebrafish guts are driven by drift and dispersal and not, for example, by selection of particularly beneficial microbes. Using the Sloan neutral community model for prokaryotes (Sloan et al. 2006), they found that, though there was some evidence of selectivity in older fish, at all ages the neutral model explained a large part of holobiont membership.

Another example of the ecological approach to understanding holobionts comes from corals and their associated microbes. Hester et al. (2016) turned to the paradigmatic holobiont of corals and their microbial communities and explored their stability and association with a new field study and a literature review. They looked at bacteria associated with three kinds of coral and two of algae from several different Pacific islands using a statistical technique they developed called the abundance–ubiquity (AU) test. This test allows a determination of microbes regularly versus sporadically associated with specific host species. They found support for the holobiont perspective that microbes are ecological assemblages on the macro-organism, and no support for a hologenome concept, because of the fluidity of association, and the lack of any control of conflict (Hester et al. 2016).

These two examples and many others like them make it clear that complex holobionts can be viewed as ecological communities. Theory suggests that stability of these communities is actually more likely to arise from competitive than cooperative interactions because cooperative interactions may lead to destabilizing positive feedbacks (Coyte et al. 2015). It is not yet clear how well supported this theory is, but disentangling cooperative from competitive interactions among members of the microbial community in hosts is an important and active area of research (McNally and Brown 2016; Stacy et al. 2016).

We have argued that strong fidelity and co-transmission are usually important for evolving organismality, but are not sufficient; there also need to be conflict-reducing mechanisms. If this is so, might it not be possible that strong conflict-reducing mechanisms alone might suffice? But if proponents of organismal holobionts want to argue this, their task is to propose and demonstrate that such mechanisms exist and that they are powerful. One possibility, though we do not think it is very likely, would hinge on a difference between a forest and a holobiont. In the holobiont, but not the forest, there is a single very large and potentially dominant partner that provides the environment for the microbes. Is it possible that this gives it sufficient power to select its microbial partners and control selfishness in them? This argument faces the problem that microbes can evolve so much more rapidly than their hosts. Moreover, even if the host were successful, this might make the microbes more like slaves or dupes than parts of an organism.

The holobiont is defined by spatial criteria. There is no reason to believe that spatial proximity necessarily leads to functional integration. After all, the malarial parasite and its host are spatially associated. The extent to which a complex holobiont is a cooperative grouping, including how much competition and conflict occur, must be decided by research but there is little reason to think they will be purely cooperative (Douglas and Werren 2016; Hester et al. 2016; Leggat et al. 2007; Moran and Sloan 2015).

Conclusions

The organism is one of the fundamental concepts in biology. It is sometimes identified with the concept of individuality but it demands something beyond what defines physical individuals. Essentially all definitions of an organism invoke some kind of physiological integration. Ours refines this by specifying that it is an adaptive unit in which all the parts interact cooperatively and not competitively. Organisms can therefore occur at multiple levels, as single cells, as cells within larger cells, as groups of cells, or even as groups of individuals.

The canonical major egalitarian transitions led to cellular groups of replicators and to the eukaryotic cell from several ancestral cells. These transitions occurred long ago but they both suggest that new levels of egalitarian organismality may require some co-transmission across generations and mechanisms to reduce conflicts. We suggest that there may be other egalitarian transitions and that certain endosymbiotic alliances are the most plausible candidates. But obviously not all multi-species groups qualify as organisms. Complex holobionts like a human and its gut microbiome are interesting for many reasons, but they are unlikely candidates for organismal function. The great majority of mutualistic associations probably fall in this class. They show some cooperation, which can even be quite extensive, but they should not be viewed as organismal unless conflicts have been reduced to the very tiny levels seen in canonical organisms.

Thus, documented biological examples akin to Theseus's fish seem to be very rare. In this view Theseus's chimeric fish is not really a fish and it is even not an organism. It remains an assemblage of multiple organisms. Why is this so given that many would agree that Theseus's ship does remain a ship even when its parts have been replaced and re-purposed from other sources? The key lies in the re-purposing. This requires a re-purposer, which for the ship would be the humans who use a tent to make a new sail and a plow to fashion a new rudder. For organisms, the only re-purposer is natural selection, and natural selection does not have the goal, as the humans do, of meshing the purposes of the new and old parts. It happens sometimes, but usually only partially, as in most mutualisms. But to get a complete transition to a new kind of combined organism seems to require very special conditions such as co-transmission and mechanisms that suppress conflict.

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