

Multispecies individuals

Pierrick Bourrat^{1,2,3}  · Paul E. Griffiths³

Received: 9 May 2017 / Accepted: 5 April 2018
© Springer International Publishing AG, part of Springer Nature 2018

Abstract We assess the arguments for recognising functionally integrated multi-species consortia as genuine biological individuals, including cases of so-called ‘holobionts’. We provide two examples in which the same core biochemical processes that sustain life are distributed across a consortium of individuals of different species. Although the same chemistry features in both examples, proponents of the holobiont as unit of evolution would recognize one of the two cases as a multi-species individual whilst they would consider the other as a compelling case of ecological dependence between separate individuals. Some widely used arguments in support of the ‘holobiont’ concept apply equally to both cases, suggesting that those arguments have misidentified what is at stake when seeking to identify a new level of biological individuality. One important aspect of biological individuality is evolutionary individuality. In line with other work on the evolution of individuality, we show that our cases can be distinguished by focusing on the fitness alignment between the partners of the consortia. We conclude that much of the evidence currently presented for the ubiquity and importance of multi-species individuals is simply not to the point, at least unless the issue of biological individuality is firmly divorced from the question of evolutionary individuality.

✉ Pierrick Bourrat
p.bourrat@gmail.com

Paul E. Griffiths
paul.griffiths@sydney.edu.au

¹ Department of Philosophy, Macquarie University, North Ryde, NSW 2109, Australia

² School of History and Philosophy of Science, University of Sydney, Main Quadrangle A14, Sydney, NSW 2006, Australia

³ Department of Philosophy and Charles Perkins Centre, University of Sydney, Main Quadrangle A14, Sydney, NSW 2006, Australia

Keywords Individuality · Evolutionary transitions in individuality · Holobiont · Symbiosis

1 Introduction

This article examines an influential class of arguments for the existence and importance of biological individuals whose component parts are from different species. We show that there is a common form to these arguments and suggest that arguments of this form are insufficient to establish their intended conclusion, namely that a multi-species consortium is a new biological individual rather than several distinct biological individuals in ecological relationships. Recently Skillings (2016) has proposed a convergent analysis with ours. Similar criticisms of the ‘holobiont’ concept have been made in the recent scientific literature. We identify an underlying problem with what we term ‘part of the system’ arguments concerning biological individuality and conclude that, as many biologists and philosophers have argued in the past, the fundamental issue in identifying new levels of biological individuality should be whether some entity can function as a unit of evolution, which will depend on the fitness alignment between the partners over evolutionary timescales.

Examples of symbioses between different species that are so tightly integrated as to seem like a new type of biological individual have long been known (e.g. lichens).¹ However, two empirical developments have led to increased interest in this phenomenon. The first was the acceptance of the theory that eukaryote cells are derived from multiple ancestral lineages (Margulis 1970 and see Sect. 5). The second was the discovery that multicellular organisms depend on an associated community of microbes in more substantial and specific ways than had previously been realized. This second development led to the idea that the biologically significant units of multicellular life are not traditional multicellular organisms—functionally integrated populations of cells descended from one or a few closely related cells—but symbiotic communities of cells from many species, or ‘holobionts’. The term ‘holobiont’ (Margulis 1991, 2) was invented to suggest a greater degree of integration, in both physiology and evolution, than the older, more general notion of symbiosis. It refers to a biological unit consisting of a traditional multicellular organism and the unicellular organisms associated with that organism that make a substantial contribution to its ability to survive and reproduce (e.g. Rosenberg and Zilber-Rosenberg 2013, 1–7). O’Malley and Dupré (2007) use the handy term ‘macrobe’ to refer to traditional multicellular organisms, making a holobiont equal to a macrobe and associated microbes.

In this paper we will use a related but broader notion of a ‘multispecies individual’. This notion is needed to encompass both holobionts and associations in which partners are of similar sizes. Although it is important to emphasize that most macrobes interact in very important ways with microbes this type of interaction is

¹ Following Rosenberg and Zilber-Rosenberg (2013, 4), by “symbiosis” we mean mutualistic relationship between two or more partners. The examples we use in this article will involve partners that live in close proximity.

not fundamentally different from some interactions between macrobes and macrobes or between microbes and microbes as recognised by leading advocates of the holobiont concept Eugene Rosenberg and Ilana Zilber-Rosenberg (2013, 4). We are not aware of any theoretical principles in evolution or ecology that distinguish macrobe-microbe interactions from these other inter-species interactions. Given this, we can either generalize the concept of the holobiont to mean any multispecies individual or we can keep that term in its current sense and use the term ‘multispecies individual’ for the more general category, reserving the term ‘holobiont’ for a macrobe with its microbiota. We choose the second option, which avoids introducing any ambiguity in the use of ‘holobiont’. However, although our arguments could in principle be applied to any case of multispecies interactions upon which the Darwinian apparatus can operate, we will mostly use examples from the holobiont literature. Finally, by ‘multispecies individual’ we mean ‘multispecies *evolutionary* individual’. While we recognize that the notion of individuality has been approached from different perspectives (physiology, immunology, developmental biology, etc.) it is clear that the advocates of the holobiont concept discussed below are also concerned with evolutionary individuality. It is this aspect of individuality that we will target with our criticisms.²

In the next section we sketch the case for the importance of holobionts. In the following section we describe two examples of potential multispecies individuals. These examples are strikingly similar, but one is a holobiont, that is a multicellular organism interacting with unicellular organisms and would be considered as an individual by proponents of the holobiont concept, while the other is not for it involves interactions between two multicellular organisms and some abiotic components of the landscape, as well as some unicellular organisms. *Prima facie*, our second example therefore appears to be a typical set of ecological interactions between distinct species. In Sect. 4 we examine some popular arguments for regarding the holobiont as a biological individual in more detail and show that they apply equally to our two examples. In the final two sections we draw on the wider scientific and philosophical literature on biological individuality to identify the difference between our two cases. We argue that the greater alignment of fitness between the different partners of a consortium allows us to distinguish these two cases. In light of this we suggest that arguments reviewed earlier in the paper do not speak to the question of whether holobionts are evolutionary individuals.

² Note that there exists a literature which understands ecosystems as individuals (for reviews see Huneman 2014a, b; Van Baalen and Huneman 2014). As recognized by Huneman (2014a), this notion of individual is different from the notion of individuality typically used in evolutionary biology, the latter of which is the focus of our analysis. For that reason, we will not discuss the view of ecosystems as individuals, although we recognize that some connections between the literature on holobionts and ecosystems as individuals exist.

2 The holobiont and ‘part of the system’ arguments

The class of arguments that we target in this paper have this form: such-and-such components are essential to the functioning of some larger system, therefore those components are part of that system. Enthusiasts for the holobiont concept have presented anatomical, physiological, developmental, immunological, and evolutionary versions of this argument in which the parts are the partners entering the symbiosis, and the system is the holobiont:

Individuals can be defined anatomically, embryologically, physiologically, immunologically, genetically, or evolutionarily [... A]ll classical conceptions of individuality are called into question by evidence of *all-pervading symbiosis*. (Gilbert, Sapp, and Tauber 2012, 325; emphasis added)

Gilbert et al. are correct that each of these definitions of individuality has been important in the history of biology, and each of them is alive and well today. In each case, the notion of a structured whole is present but with a different emphasis on what that whole is or how it is maintained. In the anatomical and physiological cases, an individual is a complex whole whose parts cooperate for the good of the whole and which involves a division of labour between those parts. In the developmental case an individual is the organized structure that undergoes concerted changes between two generations. In the case of immune individuality, a complex whole is maintained by an immune system that rejects anything that is not ‘self’. In the case of genetics, the individual is a collective with a common genome. Finally, an evolutionary individual is a collection of parts that is selected as a whole.

Each of these approaches to the individuality of macrobes, Gilbert et al. claim, have been challenged by new discoveries about the functional integration of macrobes and microbes. The unit of anatomical and physiological function is larger than the traditional organism. The unit of immunological identity is not the traditional collective of more or less genetically identical cells. In the case of biological development,

Microbial symbionts appear to be a normal and necessary part of the life cycle of all mammals [...] the symbionts are integrated into the normal networks of animal development, interacting with the eukaryotic cells of their ‘host’. (Gilbert, Sapp, and Tauber 2012, 328)

These developments, they argue, undermine the genetic approach to individuality and also support a revised evolutionary approach to individuality that recognizes the holobiont as an important unit of selection. Similar arguments are found in other authors. Key advocates for the holobiont concept Rosenberg and Zilber-Rosenberg summarise their case like this:

The major arguments for considering the holobiont as a level of selection are the following.

1. All multicellular organisms contain abundant and diverse microbiota. Often, the number of microbial cells and the sum of their genetic information are above that of their host.
2. Not only the host genome but also the microbiome can be transmitted between generations with reasonable fidelity and thus maintains [*sic*] the unique properties of the holobiont.
3. Microbiotas and their hosts interact in a manner that affects the fitness of the holobiont in many ways, including its morphology, development, behavior, physiology, and resistance to disease. Taken together, these interactions characterize the holobiont as a single and unique biological entity [...]. (Rosenberg and Zilber-Rosenberg 2016, 1)

Point 1 corresponds to what Gilbert et al. say about anatomical individuality, and point 3 reiterates those author's claims about anatomy, development, physiology (adding behavioural function), and immunology. Point 2 might be read as making a 'part of the system' argument about heredity, but it is better understood as addressing some of the concerns about evolutionary individuality that will occupy us in later sections of this paper, so we will put it aside for now. The key point we wish to draw attention to with this quotation is that leading advocates of the holobiont present 'part of the system' arguments as reasons to accept that holobionts are genuine biological individuals, where that status is taken to include them as functioning as a unit of evolution.³ A key contention of this paper is that those arguments do not support that conclusion.

The 'part of the system' form of argument has also been used to argue for 'Developmental Systems Theory', the idea that the units of development and evolution are organism-environment systems (Griffiths and Gray 1994; Griffiths and Tabery 2013; for a critical perspective see Gilbert 2003). But 'part of the system' arguments must be used with care. Many organisms depend on the earth's gravitational field for normal development: the earth's mass is a "normal and necessary part of the life cycle". However, as far as we are aware advocates of the holobiont concept do not advocate a 'hologeobiont' concept! The mere existence of functional integration between an organism and some property of our planet, or between organisms of different species, is not enough to demonstrate the existence of a new, collective individual (for a similar view in different terms see Moran and Sloan 2015, Box 1, misconception 5). In the next section we use two contrasting examples of functional integration between individuals of different species to explore what else is needed.

³ See also Dupré and O'Malley (2009, 13) quoted in Sect. 4 below.

3 Holobionts and ecological communities: two examples

The problem posed by the holobiont concept, and more generally by the concept of multispecies individuality, is that it is always open to us to consider the partners not as parts of a new, collective individual, but as individuals from more than one species that have close ecological relationships to one another (Douglas and Werren 2016).

Take the controversial example of the human gut microbiome. Should we consider a human and its microbiota as a cohesive whole that natural selection is acting upon, or should we consider them as ecological interactants? If we take the latter, more conservative approach, should we consider the local population of one bacterial species as an individual interacting with its host and with local populations of other bacterial species, or should we regard the whole microbiota or some large part of it as one individual interacting with its metazoan host? It should be noted that the question of individuality does not necessarily require an all-or-nothing answer. It is perfectly possible to work with a notion of degree of individuality (Godfrey-Smith 2009, 2013; Queller and Strassmann 2009). The human + gut microbiome holobiont might have a good claim to be considered a multispecies individual despite standing lower on the scale of individuality than a unicellular eukaryote. But even recognizing that intermediate positions are possible, we need to know what makes a clear multispecies individual in order to decide the less clear-cut cases.

To address this question we use two biological systems, each involving multiple species and each resembling the other closely in terms of metabolism. The first case is a clear case of a holobiont since it involves a multicellular organism with a microbiota within it. The second case is not a holobiont, since at least some of the partners do not have a host-symbiont relationship. They are separate lineages interacting ecologically in a landscape parts of which are essential components of the metabolic system.

The first of the two systems involves the gutless worm *Olavius algavensis*. This small worm belongs to the same class as the earthworm (Oligochaeta), measures 0.2 mm in diameter and between 20 and 30 mm in length, is found on the seafloor of the Mediterranean, and hosts a number of symbiotic bacteria (Dubilier et al. 2001; Ruehland et al. 2008). Similar systems involving other hosts of the genus *Olavius* have been described (Blazejak et al. 2005; Ruehland et al. 2008). The worm has a number of extracellular symbionts that live just under the surface of its cuticle. A comparative 16S rRNA sequence analysis revealed that up to five phylotypes of symbionts were present in *O. algavensis* (Ruehland et al. 2008) and up to six in some other species of the genus (Dubilier et al. 2005; Ruehland et al. 2008). The transmission of the symbionts from one generation of worm to the other is thought to be a combination of vertical transmission from the parent to the eggs through the rupture, at the moment of the deposition of the eggs, of so called ‘genital pads’ that contain the symbionts (Giere and Langheld 1987) and horizontal invasion of the eggs by free living bacteria from the surroundings (Dubilier et al. 2005).

Two main functions of the symbionts performed by different phylotypes have been identified, both of which involve the sulphur cycle (Dubilier et al. 2001).⁴ Some symbionts are sulphate-reducing bacteria (for more on this type of bacteria see Muyzer and Stams 2008): they take sulphate molecules and transform them into sulphide. In this process of reduction, electrons are transferred from organic hydrogen and carbon found in the environment or produced internally by *Olavius* and given by the sulphate-reducing bacteria to sulphate which is thus transformed into sulphide (Dubilier et al. 2001; Kleiner et al. 2012). This liberates energy that these bacteria can use to survive and reproduce. Sulphide is toxic to most metazoans but not to *Olavius* which, thanks to another type of symbiotic bacteria, oxidizes the sulphide back into sulphate. This reaction also liberates some energy that is used to fix carbon. The molecules produced are then used by the host and the sulphate-oxidizing bacteria to continue the cycle (see Fig. 1). Since both types of endosymbionts live in different environments (reducing for the sulphate-reducing bacteria and oxidizing for the sulphide-oxidizing bacteria) it is speculated that the worm moves sequentially between different environments so that both endosymbionts can grow, and also that the sulphide-oxidizing bacteria produce organic molecules (sugars) usable by the worm (Dubilier et al. 2001).

The sulphate-reducing endosymbiont gets sulphate and transforms it into sulphide through a reduction reaction with the reducing agents being organic molecules of carbon or hydrogen found in the environment or produced by the metabolism of the worm. The energy liberated is used by the sulphate-reducing endosymbiont to produce ATP. The sulphide produced is then oxidized into sulphate by the sulphide-oxidizing endosymbiont in the presence of oxygen. The energy produced by the sulphide-oxidizing endosymbiont is used to synthesize organic molecules (sugars) that are then used by the worm and the sulphide-oxidizing endosymbiont. (Adapted from Dubilier et al. 2001).

The second system involves four partners. Seagrass meadows are found all around the globe. One puzzle surrounding them is their ecological success. Their growth should be inhibited by the fact that seagrass meadows reduce currents which makes the seabed poor in oxygen and leads to the accumulation of organic matter (van der Heide et al. 2012). This low-oxygen environment is a perfect habitat for sulphate-reducing bacteria that use sulphate (very common in this environment) as an electron acceptor (oxidant) instead of oxygen. But, as we saw with the *Olavius* system, sulphate-reducing bacteria produce sulphide, which therefore accumulates in the sediment. The problem here is that sulphide is toxic for the seagrass when its concentration is high (Koch and Erskine 2001; van der Heide et al. 2012). One way the seagrass can reduce the level of sulphide in its immediate environment is by the transport of the oxygen created during photosynthesis to the root of the seagrass. Some of this oxygen is released into the sediment (Calleja et al. 2007; Pedersen et al. 1998). Once in contact with the oxygen the sulphide is transformed into sulphate (Koch and Erskine 2001). But that mechanism is insufficient to reduce the

⁴ For an overview of this cycle see Sievert et al. (2007).

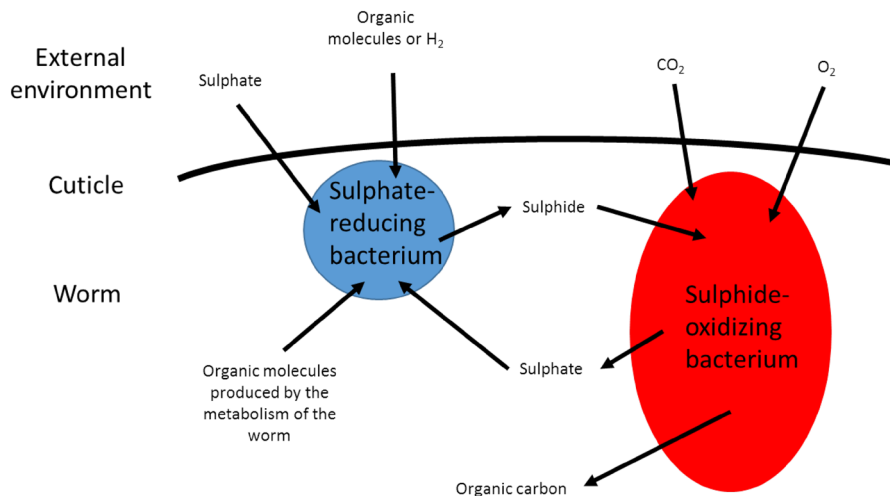


Fig. 1 Representation of the sulphur cycle in *O. algarvensis*

level of sulphide enough so that seagrass can grow or even in some cases survive, especially in warmer waters (Reynolds et al. 2007; Calleja et al. 2007).

Bivalves of the Lucinidae family are often found in the seagrass meadow ecosystem (especially around the roots of the seagrass) in mild to warm regions and it has been hypothesized that they play a crucial role in solving the problem (van der Heide et al. 2012). The reasons why these bivalves are found in these sulphide rich environments is, first, that the seagrass provides protection for the bivalves (Reynolds et al. 2007) and, second, that bivalves all have endosymbiotic sulphide-oxidizing bacteria, similar to those of *Olavius*, which live in the gills of the bivalve. These bacteria transform sulphide into sulphate in a reaction of oxidation and in the process produce sugar by fixing carbon. Finally, the bivalves benefit from the oxygen released by the seagrass in the sediments around the roots. The benefits for the bivalves are clear: they have protection and food in an environment that is hostile for most predators. Reynolds et al. (2007) hypothesize that the seagrass benefits from the action of the bivalves and their endosymbionts, which reduce the level of the toxic sulphide in the sediments and thus promote seagrass growth. This hypothesis has been confirmed experimentally, in the field and by a meta-analysis of 84 studies by van der Heide et al. (2012). An illustration of the model proposed by Reynolds et al. (2007) and van der Heide et al. (2012) is presented in Fig. 2.

The sulphate reducing bacterium in the sediment transforms sulphate into sulphide through a reduction reaction with the reducing agents being organic molecules of carbon or hydrogen found in the environment. The energy liberated is used by the sulphate-reducing bacterium to produce ATP. The sulphide produced is then oxidized into sulphate by a sulphide-oxidizing endosymbiont of a lucinid bivalve in the presence of oxygen. This process happens in the gills of the bivalve. The energy produced by the sulphide-

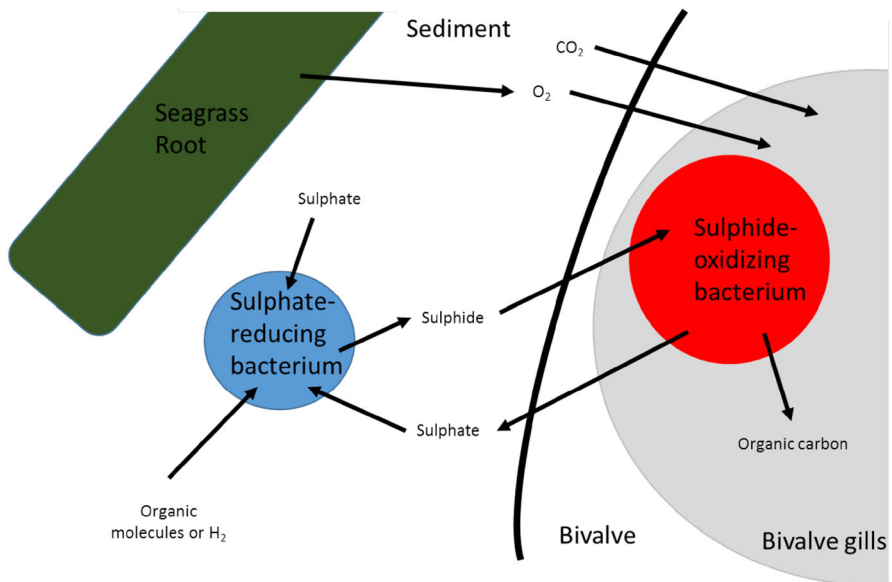


Fig. 2 Representation of the sulphur cycle in the seagrass system

oxidizing endosymbiont is used to synthesize organic molecules (sugars), which are then used by the bivalve and the sulphide-oxidizing endosymbiont.

As in the *Olavius* example, each partner in the ecological interaction depends on the others. The seagrass can grow thanks to the action of the bivalves and their endosymbionts. The bivalves can survive and reproduce thanks to the action to the seagrass that provides protection. The sulphate-reducing bacteria can survive thanks to the decomposition of organic matter and lack of current created by the presence of the seagrass. Finally, the sulphide-oxidizing endosymbionts benefits from the production of sulphide from the sulphate-reducing bacteria and from the oxygen brought to the gills of the bivalve. Yet, contrary to the case of *Olavius*, and in spite of the same function being performed, the seagrass system is not a holobiont since the seagrass and the bivalve do not have a host-symbiont relationship. In fact, the seagrass system seems to be a straightforward example of an ecosystem in which multiple species interact with one another and with abiotic factors in a reasonably stable fashion.

In the next section, we revisit in more detail the arguments that we sketched in Sect. 2. As we demonstrate, many of these arguments apply *both* to *Olavius* and to the seagrass system and thus fail to distinguish multispecies individuals, defined broadly, from ecosystems.

4 Revisiting arguments for the holobiont

We saw in Sect. 2 that many arguments used to support the holobiont concept are ‘part of the system’ arguments: certain components are essential to the functioning of a larger system and so they should be considered as part of that system. Perhaps the most straightforward version argues that a putative multispecies individual is an autonomous metabolic unit when it is considered as a whole, whilst each partner considered individually is dependent on the others for key metabolic functions. For instance, Dupré and O’Malley write that: “paradigmatic multicellular organisms [are] more multicellular than is usually supposed (in that a multicellular organism should be understood as including all the entities that interact to achieve shared metabolic and reproductive goals)” (Dupré and O’Malley 2009, 11). Later, using Hull’s (1980) famous distinction between replicator and interactor, they claim: “[i]nteractors, in our view, are 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.” (2009, 13). Their point, as we understand it, is that metabolism, defined as the ability to process external resources so as to maintain the system out of equilibrium with its environment, is a key property of units of life.⁵ Gilbert et al. put the point in terms of the contribution of phylogenetically and genetically diverse partners to a single common, physiological good—“we may conclude that on classical physiological grounds, animals are not individuals” (Gilbert et al. 2012, 330). The conclusion drawn by both sets of authors is that multispecies collectives with a single metabolism should be treated as individuals, and evolutionary theory should focus on these metabolic units.

How does the metabolic argument fare when applied to our examples? In the case of *Olavius*, it is obvious that the partners involved in the transformation do not take in raw materials and process them self-sufficiently: each partner needs the other for the whole to be sustained. The problem is that this argument also applies to the seagrass system. Although the seagrass system is a functionally integrated metabolic unit, it is inconsistent with the definition of the holobiont for the different ‘partners’ live freely in a landscape interacting with one another, and differently with the abiotic components of that landscape, as opposed to unitarily in the case of *Olavius*. Perhaps metabolic unity is a necessary condition for individuality understood evolutionarily, but it does not seem to be a sufficient one.

Ereshefsky and Pedroso confronted this problem when arguing that biofilms are multispecies individuals (Ereshefsky and Pedroso 2013). They made use of an additional criterion that they derive from Hull’s original account of interactors. An interactor “must be a cohesive whole [whose] interaction with the environment must have a unitary effect on its constituent replicators” (2013, 343). This, at first glance seems to resolve our problem. The sea grass system is less cohesive than *Olavius*. But the notion of cohesiveness is a qualitative notion, and it is not clear that quantifying it would lead to the same conclusion. For instance, if cohesiveness was measured at a particular scale as the number of causal interactions between the different partners in the system, it might be higher in the case of the seagrass system

⁵ For O’Malley’s more recent views on the topic of biological individuality, see O’Malley (2016).

than with *Olavius*. The second idea, that of unitary effect, derives from the literature on the unit of selection (for a systematic review Lloyd 2012). It seems clear that in both cases each partner is necessary for the perpetuation of the whole system, so the unitary effect criterion is met in both cases. If it is possible that there will exist a difference in specificity between the partners in the different systems, in the current state of the research, this question cannot yet be answered. Note also that the notions of ‘unitary effect’ or ‘unit of selection’ are as vague as ‘cohesiveness’ if not quantified. Thus, although the notion of an interactor is useful in certain contexts within evolutionary theory (Godfrey-Smith 2000), it is of very limited value in deciding whether a multispecies entity is an individual in its own right. These remarks should not be read as a criticism of Ereshefsky and Pedroso, who are fully aware that the notion of interactor is not satisfactory as it stands and work to add precision to the concept. Our proposal at the end of this paper follows the direction they have initiated.

The *developmental* argument for considering the holobiont as an individual closely parallels the metabolic argument. It also closely resembles earlier arguments for ‘Developmental Systems Theory’ (Griffiths and Gray 1994). Many aspects of metazoan development cannot be completed in the absence of symbiotic bacteria, as evidenced by abnormal development in gnotobiotic (‘germ-free’) experimental organisms.⁶ These essential microbial interactants are sometimes acquired through evolved, exogenetic heredity mechanisms. These facts are thought to show that “[w]e are not individuals from the viewpoint of developmental biology” (Gilbert et al. 2012, 328). How does this argument fare when applied to our examples? From the biology of the two systems, which we detailed in the previous section, it is clear in each case that each partner strongly depends on the others and that removing one partner would lead to the demise of the system.⁷ It seems to us that there is a critical distinction between the claim that “[m]icrobial symbionts appear to be a normal and necessary part of the life cycle” (Gilbert et al. 2012, 328) and the claim that microbes are part of the evolved developmental system. Gilbert himself has elsewhere stressed that development is an ecological process, in which developmental systems are designed in the expectation that the environment will provide highly specific inputs to the developmental process (Gilbert and Epel 2015). One of the examples used by Gilbert and Epel is the role of kairomones—chemical traces of predators—in the development of water fleas (*Daphnia pulex*). The fact that *Daphnia* have evolved developmental mechanisms that need chemical inputs from predators does not mean that there is a flea-predator holobiont. The facts about development adduced in support of the holobiont concept are, we will argue below, only apposite when additional conditions are met, conditions that suggest that the collective is acting as a cohesive unit of evolution.

⁶ While the adjective gnotobiotic is often used to refer to germ-free animals, it is a more inclusive term that refers to situations where we know all the microorganisms on or in an organism, including cases where there are none at all (see Rooks and Garrett 2016, Box 2).

⁷ As pointed out by the Staffan Müller-Wille, one difference between the two systems might be in the specificity of associations between the partners. As mentioned earlier, this question remains an empirical one.

Another argument for considering a holobiont as an individual is immunological. Gilbert and collaborators propose that the immune system of a holobiont discriminates in two ways (Gilbert et al. 2012; see also Gilbert 2014). Not only does it discriminate cheaters from non-cheaters within the host, but, more importantly for Gilbert et al., it accepts some genetic lineages and excludes others, rather than excluding all other genetic lineages from the host. Gilbert and collaborators speculate that the immune system of the host is an adaptation of the holobiont when they write:

The immune system has learned through evolution which organisms to exclude and kill, and which organisms to encourage, allow entry, and support. If accepted, the symbiont can mutually participate in development and physiological processes. Moreover, it can help mediate the holobiont's response to other organisms, effectively becoming self. (Gilbert, Sapp, and Tauber 2012, 333)

The mechanism proposed by Gilbert et al. is one of assortative interaction, which has long been recognized to be one means by which substantial between-group variation in fitness can occur (Wilson and Dugatkin 1997). Thus, this immunological argument fits squarely with classical models of group selection. Ecosystems like the seagrass community can in fact be quite selective in who they admit as members. Although we are not aware of research that will allow us to directly compare the seagrass system to the *Olavius* community in this respect, many organisms secrete substances into their environments to change the composition of their ecological communities in an adaptive manner, a phenomenon known as 'allelopathy'. For example, marine algae release chemicals that suppress some planktonic species but not others (Schwartz et al. 2016). The difference between these ecological interactions and cases in which immune integration (or assortative interaction more generally) represents an emergent level of individuality must be sought elsewhere.

The various 'part of the system' arguments reviewed in this section do not permit us to distinguish the two systems. This might not represent a problem if individuality is studied from a purely metabolic, developmental or immunological perspective, and if the proponent of that perspective is prepared to admit the seagrass system and similar ecosystems as individuals. From an evolutionary perspective, however, there are good reasons to distinguish the two cases, as we will see in the next section. 'Part of the system' arguments do not distinguish them and so fail as an approach to evolutionary individuality. In the next section we review the wider literature on the definition of biological individuality and the evolution of individuality in order to locate the additional resources needed to clearly distinguish our two examples.

5 The philosophy of biological individuality

Biological individuality has received considerable attention in recent philosophy of biology. In the last 5 years no less than four edited collections on the topic have been published (Bouchard and Huneman 2013; Calcott and Sterelny 2011; Guay and Pradeu 2015; Lidgard and Nyhart 2017). This in itself is not surprising, for theoretical biology needs a well-defined concept of individuality (Clarke 2013). For example, individuality is at the heart of evolutionary theory (Hull 1980; Pepper and Herron 2008). When evolutionary biologists talk about populations they typically mean population of *individuals*. When fitness is defined as *expected number of offspring*, it is assumed that we can identify *individual* offspring. Thus, without clear criteria of individuality, standard evolutionary theory is in trouble (Clarke 2013; see also Van Valen 1989). But on surveying the literature it becomes clear that there is no consensus on how to define and identify individuality (Clarke 2010, 2013; Pepper and Herron 2008). Instead, there are several competing proposals, most of which are admitted even by their proponents to be tentative and in need of further refinement.

Nevertheless, there are some common elements in several of the more influential attempts to define individuality, such as Godfrey-Smith (2009) and Queller and Strassmann (2009). Godfrey-Smith (2009, 95) proposes three criteria: the existence of a bottleneck stage during the life cycle of the collective marking generations at that level, the existence of reproductive specialization (germ/soma lines or some equivalent), and overall integration of the collective. Queller and Strassmann (2009) propose that the hallmarks of individuality are a high level of cooperation and a lack of conflicts between the parts that make up the collective. The underlying idea for both is that individuality at some level of biological organisation requires adaptation to occur at that level of organization without disruption from a lower level. Also, rather than being all or nothing criteria, the parameters proposed by Godfrey-Smith and Queller and Strassmann are quantitative ones, leading one group of cells to be *more or less* a biological individual than another group. The higher the value of each parameter, the more that case is a paradigmatic case of individuality.

One reason individuality has taken centre stage in recent philosophy of biology is the evolution of the group selection debate. In the 1960s, 70s and 80s the main question was whether groups can be units of selection and if so, how prevalent group selection is in nature. It was broadly agreed that group selection is possible but rare. But group selection resurfaced in the 1990s and 2000s when the idea that eukaryotic cells have their origins in groups of prokaryotic cells forming endosymbioses (Margulis 1970), and that multicellular organisms have their origins in groups of unicellular organisms. What were previously thought to be paradigmatic cases of individual-level selection came to be seen as the result of earlier selection processes at the level of groups of cells (Griesemer 2000; Maynard Smith and Szathmari 1995; Michod 1999; Okasha 2006). The question of whether groups are levels or units of selection, which can be found in the work of Lloyd (1988) or Wade (2016) among others (for a systematic review see Lloyd 2012), has progressively been replaced by the question the emergence of new levels of

individuality by a process of selection at a higher level of individuality. Much work since then has been dedicated to the so called ‘major transitions in evolution’, or as they are now usually called, ‘evolutionary transitions in individuality’ (ETIs) in which the explanatory target is the transition from a group of particles to a collective that is an individual in its own right (for recent reviews of the literature see O’Malley and Powell 2016; Szathmary 2015).

Much of the literature on ETIs focused on transitions in which the higher level entity resulting from the transition is both unquestionably an individual and results from the cooperation of partners with a recent common origin, or what Queller (1997) calls ‘fraternal transitions’. Attempts to understand the processes underlying fraternal ETIs have used models systems that either did not transition (and are not expected to transition) or did fully transition. Although no actual transition in progress is observed, the idea is that systems of these two kinds, located on either side of a transition, can give insights into the process.

An illustration of the first kind of model is Rainey and collaborators, with the Wrinkly Spreader strain of the bacterium *Pseudomonas fluorescens* (Hammer-schmidt et al. 2014; Rainey and Kerr 2010; Rainey and Rainey 2003). Wrinkly Spreader is able to produce a polymer that gives the population a selective advantage by allowing them to form a mat at the surface of water. Bacteria in the mat get more oxygen than when swimming in the milieu but they incur the cost of producing the polymer that supports the mat. The system is sensitive to cheating: mutants that benefit from the mat but do not produce the polymer inevitably arise and grow. This ultimately leads to the collapse of the mat. A new mat can, however, be produced in some cases if some of the bacteria not trapped in the old mat (presumably because they did not produce the polymer) mutate in the reverse direction and start producing the polymer. Wrinkly spreader has not and is not expected to transition to multicellularity, but it seems to be hovering on the edge.

An illustration of the second kind of model is the work of Michod and his collaborators with *Volvox* (Herron et al. 2009; Michod 1999, 2005). *Volvox* is a genus of multicellular algae with some species in which individuals consist of a very small number of cells. Their transition from uni- to multicellularity is estimated to have occurred around 200 million years ago (Herron et al. 2009). Although *Volvox* has transitioned from a unicellular organism to a multicellular one, because different *Volvox* species have different numbers of cells with different degrees of specialisation (division of labour, presence or absence of cells sensitive to light, etc.), the genus *Volvox* as a whole can be used as a model of stages in a fraternal transition.

More recently another form of ETI, which Queller (1997) calls an ‘egalitarian’ transition, has received attention. In egalitarian transitions, partners have very different evolutionary origins and bring to the collective different functions. A classic example of egalitarian transition is the endosymbiotic origin of eukaryotic cells in which one prokaryotic cell engulfed another (in some lineages this process has been repeated more than once). The case of eukaryotic cells is a clear example of a transition in individuality, but several authors have proposed that collectives of particles with more fuzzy boundaries than the eukaryotic cell, and which would traditionally be considered as two or more symbiotic partners, may be individuals

(Bordenstein and Theis 2015; Dupré and O'Malley 2009; Ereshefsky and Pedroso 2013; Gilbert 2014; Gilbert et al. 2012; Mandrioli and Manicardi 2013; Singh et al. 2013). For example, Ereshefsky and Pedroso (2013) propose that multi-species communities of bacteria, such as biofilms, can be considered as individuals in their own right.⁸ Similarly, some authors have argued that the gut microbiome is a human organ or organ system (e.g., Baquero and Nombela 2012; Brown and Hazen 2015; Possemiers et al. 2011). The holobiont concept that we encountered at the beginning of this paper belongs to this tradition.

The idea of a multi-species individual that we introduced in Sect. 2 can now be defined in a new way. A multispecies individual is what emerges in a successful egalitarian ETI (for a convergent view see Estrela et al. 2016). Seen in this light our attention is directed away from the functional integration ('part of the system') arguments for multi-species individuals that fail to distinguish multispecies individuals from ecological communities, as we saw in Sect. 3. Instead, our attention is directed to the conditions that are required for an evolutionary transition in individuality.

The influential accounts of those conditions that we discussed above (Queller and Strassmann 2009; Godfrey-Smith 2009) were devised to deal with the evolution of paradigm biological individuals, such as metazoans, metaphytes and multicellular fungi. They are harder to apply to cases in which individuality arises from a symbiosis between two or more unequal partners (Godfrey-Smith 2015). But the underlying idea remains valuable: if there is to be a new level of biological individuality, then adaptation must be possible at the level of the collective without disruption from a lower level. The functional integration between different species that has been used to defend the 'holobiont' concept is only significant when it is packaged into distinct units and that packaging forces changes in fitness of one partner to lead to a proportionate change in fitness in its partners on evolutionary timescales, or in other words, when the fitness of partners is aligned.

6 Why is *Olavius* a convincing multispecies individual?

As mentioned earlier the *Olavius* system is a holobiont while the seagrass system is not. But the arguments we reviewed were unsuccessful in finding a significant difference between the two cases. We can legitimately ask whether the distinction made by the holobiont-as-unit-of-evolution proponents is not merely based on intuition. Perhaps the worm system fits our stereotype of a biological individual better than the seagrass system, but there is no theoretically significant difference between them. For example, perhaps the intuition is driven by the fact that all the bits of the *Olavius* system are biotic whilst some bits of the seagrass system are abiotic (for instance, the sediment.) This could explain why the *Olavius* and the seagrass systems produce different intuitions but it would not provide good theoretical grounds in which to anchor a concept of individuality.

⁸ Although see the recent arguments against this view by Clarke (2016).

Another obvious difference between the two systems is the sheer physical scale of what appears, intuitively, to be a single instance of each system. Although the metabolic pathways for both the seagrass system and the *Olavius* system occur at the same scale, what is considered as one instance of the seagrass system is something much larger than one instance of the *Olavius* system. A single *Olavius* worm and its symbionts is one multispecies individual, whereas a whole area of seabed in which the seagrass and its symbionts grow seems to be one multispecies individual if anything is in this case. We believe this intuitive difference in scale is a superficial manifestation of a difference in what we call *fitness boundedness* between the two systems and that fitness boundedness is a theoretically significant property.

We suggest that fitness boundedness can be captured by something like the following criterion, drawing on several recent proposals in the biological literature (see for instance Douglas and Werren 2016; Estrela et al. 2016; Moran and Sloan 2015). We also suggest this ‘fitness alignment criterion’ provides a good explication of the idea of ‘unitary effect’ proposed by Ereshefsky and Pedroso (2013), and indeed of the notion of unit of selection more generally.

(Fitness Alignment) Considering both a spatial and temporal scale at which an ensemble can be divided into units, the higher the probability that manipulating properties of one unit reliably leads to effects of the same magnitude and direction on the realized fitness of all the partners and only the partners constituting that unit at the temporal scale considered, the more highly each unit scores on *individuality*.

The fitness alignment criterion has two uses. First, it allows us to decide whether, given one particular unit, this unit is fitness bounded and is effectively a way to detect when a system has what Clarke’s (2013) calls demarcation mechanisms.’ Clarke defines these as “any mechanism that increases or maintains the capacity of an object to undergo between-object selection” (2013, 424). For instance, spatial boundedness between different units, thanks to a cuticle or membrane, might be one way to produce a unitary effect of the manipulation of one component on the whole unit. But spatial boundedness is not the only way fitness boundedness might be realised (Hull 1992; Sober 1991). We mentioned earlier that assortative interaction is one way by which variation between groups might emerge and be maintained. Groups can be spatially overlapping without interacting and consequently fitness-bounded units might exist with a low degree of spatial boundedness.⁹

Second, in the absence of a candidate unit of individuality, the criterion allows us to discover, by varying the components of a unit and observing the effects on fitness, whether some units are more fitness bounded than another, and consequently score higher on individuality. In other words, the fitness alignment criterion is an indicator of fitness-boundedness or evolutionary individuality.¹⁰

⁹ For a discussion on the mechanisms by which partners of a multispecies unit might align their fitness see O’Malley (2016).

¹⁰ This criterion can be linked to the notion of groups as equivalence classes of individuals proposed by Godfrey-Smith (2006, 2008).

Several further points should be noted about the fitness alignment criterion. First, as stated, the criterion could be satisfied at different spatio-temporal scales. This leads to a ‘nested’ view of individuality where an individual at a higher level and over a long time scale can contain a population of individuals at a lower level and over a shorter timescale. This is a view which is in some respect similar to the one proposed by Godfrey-Smith (2009). The importance of taking time scales into consideration for measures of fitness, especially in the context of ETIs, has been pointed out in Bourrat (2015b, c). Second, by ‘fitness’ we means something more general than ‘reproductive output’. A limit case of difference in fitness might be a case of difference in persistence between the units compared. For more on the concept of persistence and its relation to evolutionary concepts such as fitness and heritability see Bouchard (2008, 2011) and Bourrat (2014, 2015a). Note also that fitness refer here to a property of the candidate unit, that is ‘absolute fitness’, not a population relative property such as ‘relative fitness’

Third, the criterion is compatible with the view that individuality comes in degrees in two respects. Individuality might come in degrees in the sense that not all pairs of partners in a consortium need to have the same level of fitness alignment with one another. Individuality might also come in degrees in the sense that the fitness is more or less aligned over shorter or longer timescales. This leads us to our fourth remark which is that, as noted by Moran and Sloan (2015), even in cases where there is strong integration between the partners, conflict might still arise, as when unicellular symbionts become pathogens. For an example with nitrogen-fixing bacteria which also demonstrates the complex interaction between mutualistic and parasitic strategies in a single system see Denison and Kiers (2004). Perhaps the most extreme version of this criticism is found in Douglas and Werren (2016). Approaching the holobiont concept from an evolutionary perspective, they argue that the notion of ‘holobiont’ is too restrictive as it “is concerned with cooperative and integrative features of host-microbe systems to the exclusion of other kinds of interactions, including antagonism among microorganisms and conflicts between host and microbial partners”. Although we agree with their general approach, we believe they demand too strict a fitness alignment between partners. Although focusing on conflict is important, conflicts are pervasive even in entities that we regard as paradigmatic individuals. Our criterion, by having the form of a ‘test’, not only recognises the importance of conflicts, but also their short-term evolutionary consequences. When conflicts arise but all the partners are ultimately ‘in the same boat’, there is still scope for individuality. When at least one of the partners can escape the boat, and thus break the link between its fitness and that of its partners, there is less scope for individuality.

We now proceed to show that the fitness alignment criterion can discriminate between the *Olavius* and the seagrass systems. Let us start with the *Olavius* system. Manipulating a character of a unit would certainly affect the fitness of all the partners of a unit with the same or a very similar magnitude and in same direction. To see this, imagine for instance that we substitute one strain of sulphate-reducing bacteria by a mutant strain unable to reduce sulphate to sulphide as efficiently as before. This manipulation would reduce the fitness of every partner in the symbiosis after one generation of the worm. Depending on the strictness of the

interdependence of each partner on the others, that is the ability of one or some of the partners to tolerate the diminution or absence of the function of the other partners in the maintenance and reproduction of the unit, the manipulation might lead to effects of different magnitude and direction on the fitness of each partner. That said, the evidence we presented suggests that a partial fitness interdependence between the different partners, what Douglas and Werren (2016) call ‘partner fidelity’, exists. In fact, as we noted earlier, genital pads are adaptations that permit the vertical transmission of some of the symbionts from parents to offspring. From recent work on *Olavius algarvensis*, it appears that most symbionts are transmitted vertically, while horizontal transmission occurs occasionally only (Schimak 2016). We therefore consider that by our fitness-alignment criterion the *Olavius* system scores relatively high on individuality.

We can now see why Rosenberg and Zilber-Rosenberg included in their list of key arguments for the holobiont concept, that “[n]ot only the host genome but also the microbiome can be transmitted between generations with reasonable fidelity and thus maintains the unique properties of the holobiont” (Rosenberg and Zilber-Rosenberg 2016, 1). Their formulation is, however, ambiguous between a process that brings the same set of species together in each generation and a process that ties specific lineages of each species together. It is only the latter process that will promote adaptation at the collective level, and it is more likely to happen when individual symbioses are fitness bounded.

Things are rather different with the seagrass system since applying the fitness alignment criterion to units of this system at various spatial and temporal scales, seems to offer no reason to believe that a manipulation would, in general, lead to a similar magnitude and direction of fitness effect on each partner. Performing the same manipulation that we described for the *Olavius* case would certainly be expected to temporarily reduce the fitness of each partner in the unit of the system on which we perform the intervention. This is because the manipulation would entail that the sulphide-oxidizing bacteria surrounding the mutant sulphate-reducing bacteria would have less food, which would also affect the bivalves. That said, because each partner in this unit also depends on other units of the system, we would expect considerable variation in the magnitude and direction of the effects on the fitness of the different partners of the focal unit. Some partners would suffer a lot from the manipulation while others would be almost unaffected. Only if there are mechanisms that prevent the effects of manipulating the character of one unit to have different (or dissimilar) effects on sub-unit entities within and outside that unit can the fitness of the different partners be kept aligned. One obvious way this can be realised is by the existence of a physical boundary separating all the partners of a unit from an external milieu. However, as we mentioned earlier, this is not be the only way. Other forms of assortative interaction can be drivers of individuality (Clarke 2013; O’Malley 2016).

The alignment of fitness between partners in the seagrass system probably differs as we change the spatial and temporal scale of the units. We might expect the highest level if we treated each entire contiguous stretch of sea-grass meadow as a unit. Nevertheless, even here the extent to which the fitness of every partner is affected equally by manipulating one partner seems unlikely to equal that in a single

Olavius worm system. Furthermore, it seems likely that the fitness alignment between the different partners would be lower when considering the complete seagrass system, rather than the bivalve and its symbionts as a unit independently from the seagrass. The bivalve and its endosymbionts alone might have a comparable score to the *Olavius* system, depending on issues such as fidelity of partner transmission.

Our comparison of the *Olavius* and seagrass systems suggests that functional integration between multiple species is not significant unless it leads to an alignment of the fitness of the different partners involved in the interaction. Another way to look at this conclusion is as locating the missing step in the ‘part of the system’ arguments that we criticized above (Sect. 5). Recall that these arguments urge that, because something is essential to the integrity of a system, then it should be considered part of that system. Such arguments, whether physiological, developmental or immunological, are supposed to show that what was previously considered an individual’s environment or its ecological interaction partner, is in fact part of a larger individual. We can now see that the extra component needed to complete such an argument is that the ‘external’ element of the putative new individual is not equally part of many other putative individuals, so that its evolutionary success is linked to one particular putative new individual. When this condition is not met, then ‘part of the system’ considerations demonstrate only that biological individuals are not autonomous from their surroundings.¹¹

7 Conclusion

The primary aim of this paper was to criticise an influential class of arguments for the existence and importance of ‘multi-species individuals’—biological individuals whose component parts are from different species. As we showed, authors who advance these arguments take biological individuality to include *evolutionary* individuality. Our criticisms target this class of arguments when they are used in support of *evolutionary* multispecies individuals. We showed that there is a common form to the anatomical, physiological, immunological and developmental arguments for recognising multi-species individuals. They are all ‘part of the system’ arguments: such-and-such components are essential to the functioning of some larger system, therefore those components are part of that system. We showed that these arguments apply equally to the two systems we presented, the *Olavius* system and the seagrass system. However, although both systems make a living from the same distributed metabolism, only one would generally be considered as a holobiont, that is a new multi-species individual. We concluded that ‘part of the system’ arguments are not sufficient to establish their intended conclusion, namely that a multi-species consortium is a new evolutionary individual rather than several distinct biological individuals in ecological relationships.

¹¹ For an extended discussion of how Developmental Systems Theorists can bridge this missing step in the argument, see Griffiths and Stotz (2018).

In the later part of the paper we suggested that the evidence concerning anatomical, physiological, immunological and developmental integration marshalled by proponents of multi-species individuality is only relevant when additional conditions are met, conditions that suggest that the multi-species collective is acting as a cohesive unit of evolution. We reviewed the extensive philosophical and scientific literature that identifies this as the real question concerning multi-species individuality and presented a criterion of individuality that draws on this literature, the ‘fitness alignment criteria’. We showed that the *Olavius* system satisfies this criterion more convincingly than the seagrass system.

If the arguments presented here are correct, much of the evidence currently presented for the ubiquity and importance of multi-species individuals is simply not to the point, at least unless the issue of biological individuality is firmly divorced from the question of evolutionary individuality. We hope this paper will contribute to shifting the focus of the literature on individuality from findings about anatomical, physiological, immunological and developmental interdependence, however novel and surprising these may be, to concentrate more on documenting the integration of evolutionary interests between partners. This is a trend we can observe in the biological literature on this topic, and which will hopefully soon be seen in the philosophical literature.

Acknowledgements We are thankful to Maxime Aodr e, Brett Calcott, Guilhem Doucier, Andrew Holmes, Arnaud Pocheville, Steven Quistad, Paul Rainey, Karola Stotz and Elena Walsh for their comments on a previous version of the manuscript. We are particularly thankful to Andrew Holmes and Arnaud Pocheville for discussions on this topic. This research was supported under Australian Research Council’s Discovery Projects funding scheme (Project DP0878650).

References

- Baquero, F., & Nombela, C. (2012). The microbiome as a human organ. *Clinical Microbiology & Infection*, 18, 2–4. <https://doi.org/10.1111/j.1469-0691.2012.03916.x>.
- Blazejak, A., Christer, E., Rudolf, A., & Nicole, D. (2005). Coexistence of bacterial sulfide oxidizers, sulfate reducers, and spirochetes in a gutless worm (*Oligochaeta*) from the Peru Margin. *Applied and Environmental Microbiology*, 71(3), 1553–1561. <https://doi.org/10.1128/AEM.71.3.1553-1561.2005>.
- Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLoS Biology*, 13(8), e1002226. <https://doi.org/10.1371/journal.pbio.1002226>.
- Bouchard, F. (2008). Causal processes, fitness, and the differential persistence of lineages. *Philosophy of Science*, 75(5), 560–570.
- Bouchard, F. (2011). Darwinism without populations: A more inclusive understanding of the ‘survival of the fittest’. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, 42(1), 106–114.
- Bouchard, F., & Huneman, P. (Eds.). (2013). *From groups to individuals: Evolution and emerging individuality*. Vienna series in theoretical biology Cambridge, MA: The MIT Press.
- Bourrat, P. (2014). From survivors to replicators: Evolution by natural selection revisited. *Biology and Philosophy*, 29(4), 517–538.
- Bourrat, P. (2015a). How to read ‘heritability’ in the recipe approach to natural selection. *British Journal for The Philosophy of Science*, 66(4), 883–903.
- Bourrat, P. (2015b). Levels, time and fitness in evolutionary transitions in individuality. *Philosophy & Theory in Biology*. <https://doi.org/10.3998/ptb.6959004.0007.001>.

- Bourrat, P. (2015c). Levels of selection are artefacts of different fitness temporal measures. *Ratio*, 28(1), 40–50. <https://doi.org/10.1111/rati.12053>.
- Brown, J. M., & Hazen, S. L. (2015). The gut microbial endocrine organ: Bacterially derived signals driving cardiometabolic diseases. *Annual Review of Medicine*, 66(1), 343–359. <https://doi.org/10.1146/annurev-med-060513-093205>.
- Calcott, B., & Sterelny, K. (Eds.). (2011). *The major transitions in evolution revisited.*, Vienna series in theoretical biology Cambridge, MA: MIT Press.
- Calleja, M. L., Marbà, N., & Duarte, C. M. (2007). The relationship between seagrass (*Posidonia Oceanica*) decline and sulfide porewater concentration in carbonate sediments. *Estuarine, Coastal and Shelf Science*, 73(3–4), 583–588. <https://doi.org/10.1016/j.ecss.2007.02.016>.
- Clarke, E. (2010). The problem of biological individuality. *Biological Theory*, 5(4), 312–325.
- Clarke, E. (2013). The multiple realizability of biological individuals. *The Journal of Philosophy*, 110(8), 413–435.
- Clarke, E. (2016). Levels of selection in biofilms: Multispecies biofilms are not evolutionary individuals. *Biology and Philosophy*, 31(2), 191–212. <https://doi.org/10.1007/s10539-016-9517-3>.
- Denison, R. F., & Kiers, E. T. (2004). Lifestyle alternatives for rhizobia: Mutualism, parasitism, and forgoing symbiosis. *FEMS Microbiology Letters*, 237(2), 187–193. <https://doi.org/10.1016/j.femsle.2004.07.013>.
- Douglas, A. E., & Werren, J. H. (2016). Holes in the hologenome: Why host-microbe symbioses are not holobionts. *MBio*, 7(2), e02099. <https://doi.org/10.1128/mBio.02099-15>.
- Dubilier, N., Blazejak, A., & Rühlend, C. (2005). Symbioses between bacteria and gutless marine oligochaetes. In J. Overmann (Ed.), *Molecular basis of symbiosis* (pp. 251–275)., Progress in molecular and subcellular biology 41 Berlin: Springer. https://doi.org/10.1007/3-540-28221-1_12.
- Dubilier, N., Mülders, C., Ferdelman, T., de Beer, D., Pernthaler, A., Klein, M., et al. (2001). Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. *Nature*, 411(6835), 298–302. <https://doi.org/10.1038/35077067>.
- Dupré, J., & O'Malley, M. A. (2009). Varieties of living things: Life at the intersection of lineage and metabolism. *Philosophy & Theory in Biology*. <https://doi.org/10.3998/ptb.6959004.0001.003>.
- Ereshefsky, M., & Pedroso, M. (2013). Biological individuality: The case of biofilms. *Biology and Philosophy*, 28(2), 331–349. <https://doi.org/10.1007/s10539-012-9340-4>.
- Estrela, S., Kerr, B., & Morris, J. J. (2016). Transitions in individuality through symbiosis. *Current Opinion in Microbiology*, 31, 191–198. <https://doi.org/10.1016/j.mib.2016.04.007>. (**environmental microbiology * special section: megaviromes**).
- Giere, O., & Langheld, C. (1987). Structural organisation, transfer and biological fate of endosymbiotic bacteria in gutless oligochaetes. *Marine Biology*, 93(4), 641–650. <https://doi.org/10.1007/BF00392801>.
- Gilbert, S. F. (2003). Evo-devo, devo-evo, and devgen-popgen. *Biology and Philosophy*, 18(2), 347–352.
- Gilbert, S. F. (2014). Symbiosis as the way of eukaryotic life: The dependent co-origination of the body. *Journal of Biosciences*, 39(2), 201–209. <https://doi.org/10.1007/s12038-013-9343-6>.
- Gilbert, S. F., & Epel, D. (2015). *Ecological developmental biology: The environmental regulation of development, health, and evolution* (2nd ed.). Sunderland, MA: Sinauer Associates, Inc. Publishers.
- Gilbert, S. F., Sapp, J., & Tauber, A. I. (2012). A symbiotic view of life: We have never been individuals. *The Quarterly Review of Biology*, 87(4), 325–341. <https://doi.org/10.1086/668166>.
- Godfrey-Smith, P. (2000). The replicator in retrospect. *Biology and Philosophy*, 15(3), 403–423. <https://doi.org/10.1023/A:1006704301415>.
- Godfrey-Smith, P. (2006). Local interaction, multilevel selection, and evolutionary transitions. *Biological Theory*, 1(4), 372–380.
- Godfrey-Smith, P. (2008). Varieties of population structure and the levels of selection. *The British Journal for the Philosophy of Science*, 59(1), 25–50.
- Godfrey-Smith, P. (2009). *Darwinian populations and natural selection*. Oxford, NY: Oxford University Press.
- Godfrey-Smith, P. (2013). Darwinian individuals. In F. Bouchard & P. Huneman (Eds.), *From groups to individuals: Evolution and emerging individuality* (pp. 17–36). Cambridge: The MIT Press.
- Godfrey-Smith, P. (2015). Reproduction, symbiosis, and the eukaryotic cell. *Proceedings of the National Academy of Sciences*, 112(33), 10120–10125. <https://doi.org/10.1073/pnas.1421378112>.
- Griesemer, J. R. (2000). The units of evolutionary transition. *Selection*, 1(1), 67–80.
- Griffiths, P. E., & Gray, R. D. (1994). Developmental systems and evolutionary explanation. *Journal of Philosophy XCI*, 6, 277–304.

- Griffiths, P. E., & Stotz, K. (2018). How DST became a process theory. In D. J. Nicholson & J. Dupré (Eds.), *Everything flows: Towards a processual philosophy of biology*.
- Griffiths, P. E., & Tabery, James. (2013). Developmental systems theory: What does it explain, and how does it explain it? *Advances in Child Development and Behavior*, 44, 65–94.
- Guay, A., & Pradeu, T. (Eds.). (2015). *Individuals across the sciences*. Oxford: Oxford University Press.
- Hammerschmidt, K., Rose, C. J., Kerr, B., & Rainey, P. B. (2014). Life cycles, fitness decoupling and the evolution of multicellularity. *Nature*, 515(7525), 75–79. <https://doi.org/10.1038/nature13884>.
- Heide, T., Govers, L. L., de Fouw, J., Olf, H., van der Geest, M., van Katwijk, M. M., et al. (2012). A three-stage symbiosis forms the foundation of seagrass ecosystems. *Science*, 336(6087), 1432–1434.
- Herron, M. D., Hackett, J. D., Aylward, F. O., & Michod, R. E. (2009). Triassic origin and early radiation of multicellular volvocine algae. *Proceedings of the National Academy of Sciences*, 106(9), 3254–3258.
- Hull, D. L. (1980). Individuality and selection. *Annual Review of Ecology and Systematics*, 11, 311–332.
- Hull, D. L. (1992). Individual. In E. F. Keller & E. A. Lloyd (Eds.), *Keywords in evolutionary biology* (pp. 180–187). Cambridge, MA: Harvard University Press.
- Huneman, P. (2014a). Individuality as a theoretical scheme. I. Formal and material concepts of individuality. *Biological Theory*, 9(4), 361–373. <https://doi.org/10.1007/s13752-014-0192-9>.
- Huneman, P. (2014b). Individuality as a theoretical scheme. II. About the weak individuality of organisms and ecosystems. *Biological Theory*, 9(4), 374–381. <https://doi.org/10.1007/s13752-014-0193-8>.
- Kleiner, M., Wentrup, C., Lott, C., Teeling, H., Wetzel, S., Young, J., et al. (2012). Metaproteomics of a gutless marine worm and its symbiotic microbial community reveal unusual pathways for carbon and energy use. *Proceedings of the National Academy of Sciences*, 109(19), E1173–E1182. <https://doi.org/10.1073/pnas.1121198109>.
- Koch, M. S., & Erskine, J. M. (2001). Sulfide as a phytotoxin to the tropical seagrass *Thalassia testudinum*: Interactions with light, salinity and temperature. *Journal of Experimental Marine Biology and Ecology*, 266(1), 81–95. [https://doi.org/10.1016/S0022-0981\(01\)00339-2](https://doi.org/10.1016/S0022-0981(01)00339-2).
- Lidgard, S., & Nyhart, L. K. (Eds.). (2017). *Biological individuality: Integrating scientific, philosophical, and historical perspectives*. Chicago, London: University of Chicago Press.
- Lloyd, E. A. (1988). *The structure and confirmation of evolutionary theory*. New York: Greenwood Press.
- Lloyd, E. A. (2012). Units and levels of selection. In E. N. Zalta (Ed.), *The Stanford encyclopedia of philosophy*. <http://plato.stanford.edu/archives/win2012/entries/selection-units/>.
- Mandrioli, M., & Manicardi, G. C. (2013). Evolving aphids: One genome-one organism insects or holobionts. *Invertebrate Survival Journal*, 10, 1–6.
- Margulis, L. (1970). *Origin of eukaryotic cells: Evidence and research implications for a theory of the origin and evolution of microbial, plant, and animal cells on the precambrian earth*. New Haven: Yale University Press.
- Margulis, L. (1991). Symbiogenesis and symbiogenesis. In L. Margulis & R. Fester (Eds.), *Symbiosis as a source of evolutionary innovation: Speciation and morphogenesis* (pp. 1–14). Boston, MA: MIT Press.
- Maynard Smith, J., & Szathmari, E. (1995). *The major transitions in evolution*. Oxford: OUP Oxford.
- Michod, R. E. (1999). *Darwinian dynamics: Evolutionary transitions in fitness and individuality*. Princeton, NJ: Princeton University Press.
- Michod, R. E. (2005). On the transfer of fitness from the cell to the multicellular organism. *Biology and Philosophy*, 20(5), 967–987. <https://doi.org/10.1007/s10539-005-9018-2>.
- Moran, N. A., & Sloan, D. B. (2015). The hologenome concept: Helpful or hollow? *PLoS Biology*, 13(12), e1002311. <https://doi.org/10.1371/journal.pbio.1002311>.
- Muyzer, G., & Stams, A. J. M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews Microbiology*. <https://doi.org/10.1038/nrmicro1892>.
- O'Malley, M. A. (2016). Reproduction expanded: Multigenerational and multilineal units of evolution. *Philosophy of Science*, 83, 835–847.
- O'Malley, M. A., & Dupré, J. (2007). Size doesn't matter: Towards a more inclusive philosophy of biology. *Biology and Philosophy*, 22(2), 155–191. <https://doi.org/10.1007/s10539-006-9031-0>.
- O'Malley, M. A., & Powell, R. (2016). Major problems in evolutionary transitions: How a metabolic perspective can enrich our understanding of macroevolution. *Biology and Philosophy*, 31(2), 159–189. <https://doi.org/10.1007/s10539-015-9513-z>.
- Okasha, S. (2006). *Evolution and the levels of selection*. Oxford: Oxford University Press.

- Pedersen, O., Borum, J., Duarte, C. M., & Fortes, M. D. (1998). Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*. *Marine Ecology Progress Series*, 169, 283–288.
- Pepper, J. W., & Herron, M. D. (2008). Does biology need an organism concept? *Biological Reviews*, 83(4), 621–627. <https://doi.org/10.1111/j.1469-185X.2008.00057.x>.
- Possemiers, S., Bolca, S., Verstraete, W., & Heyerick, A. (2011). The intestinal microbiome: A separate organ inside the body with the metabolic potential to influence the bioactivity of botanicals. In *Fitoterapia*, Papers from the 2010 DSHEA symposium, Chicago, IL, USADSHEA 2010 symposium Chicago, 82 (1): 53–66. <https://doi.org/10.1016/j.fitote.2010.07.012>.
- Queller, D. C. (1997). “Cooperators since life began.” Edited by John Maynard Smith and Eors Szathmáry. *The Quarterly Review of Biology*, 72(2), 184–188.
- Queller, D. C., & Strassmann, J. E. (2009). Beyond society: The evolution of organismality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1533), 3143–3155. <https://doi.org/10.1098/rstb.2009.0095>.
- Rainey, P. B., & Kerr, B. (2010). Cheats as first propagules: A new hypothesis for the evolution of individuality during the transition from single cells to multicellularity. *BioEssays*, 32(10), 872–880. <https://doi.org/10.1002/bies.201000039>.
- Rainey, P. B., & Rainey, K. (2003). Evolution of cooperation and conflict in experimental bacterial populations. *Nature*, 425(6953), 72–74. <https://doi.org/10.1038/nature01906>.
- Reynolds, L. K., Berg, P., & Zieman, J. C. (2007). Lucinid clam influence on the biogeochemistry of the seagrass *Thalassia testudinum* sediments. *Estuaries and Coasts*, 30(3), 482–490.
- Rooks, M. G., & Garrett, W. S. (2016). Gut microbiota, metabolites and host immunity. *Nature Reviews Immunology*, 16(6), 341–352. <https://doi.org/10.1038/nri.2016.42>.
- Rosenberg, E., & Zilber-Rosenberg, I. (2013). *The hologenome concept: Human, animal and plant microbiota*. Cham: Springer. <https://doi.org/10.1007/978-3-319-04241-1>.
- Rosenberg, E., & Zilber-Rosenberg, I. (2016). Microbes drive evolution of animals and plants: The hologenome concept. *MBio*. <https://doi.org/10.1128/mBio.01395-15>.
- Ruehland, C., Anna, B., Lott, C., Loy, A., Erséus, C., & Dubilier, N. (2008). Multiple bacterial symbionts in two species of co-occurring gutless oligochaete worms from Mediterranean sea grass sediments. *Environmental Microbiology*, 10(12), 3404–3416. <https://doi.org/10.1111/j.1462-2920.2008.01728.x>.
- Schimak, M.-P. (2016). Transmission of bacterial symbionts in the gutless oligochaete *Olavius algarvensis*. Bremen, Universität Bremen. <http://d-nb.info/1084866269/>.
- Schwartz, E. R., Poulin, R. X., Mojib, N., & Kubanek, J. (2016). Chemical ecology of marine plankton. *Natural Product Reports*. <https://doi.org/10.1039/c6np00015k>.
- Sievert, S. M., Kiene, R. P., & Schultz-Vogt, H. N. (2007). The sulfur cycle. <http://darchive.mblwhoilibrary.org/handle/1912/2786>.
- Singh, Y., Ahmad, J., Musarrat, J., Ehtesham, N. Z., & Hasnain, S. E. (2013). Emerging importance of holobionts in evolution and in probiotics. *Gut Pathogens*, 5(12), 1–8.
- Skilling, D. (2016). Holobionts and the ecology of organisms: Multi-species communities or integrated individuals? *Biology and Philosophy*, 31(6), 875–892. <https://doi.org/10.1007/s10539-016-9544-0>.
- Sober, E. (1991). Organisms, individuals, and units of selection. In A. I. Tauber (Ed.), *Organism and the origins of self* (Vol. 129, pp. 275–296). Boston studies in the philosophy of science Amsterdam: Springer. https://doi.org/10.1007/978-94-011-3406-4_13.
- Szathmáry, E. (2015). Toward major evolutionary transitions theory 2.0. *Proceedings of the National Academy of Sciences*, 112(33), 10104–10111. <https://doi.org/10.1073/pnas.1421398112>.
- Van Baalen, M., & Huneman, P. (2014). Organisms as ecosystems/ecosystems as organisms. *Biological Theory*, 9(4), 357–360. <https://doi.org/10.1007/s13752-014-0194-7>.
- Van Valen, L. M. (1989). Three paradigms of evolution. *Evolutionary Theory*, 9, 1–17.
- Wade, M. J. (2016). *Adaptation in metapopulations: How interaction changes evolution*. Chicago: University of Chicago Press.
- Wilson, D. S., & Dugatkin, L. A. (1997). Group selection and assortative interactions. *The American Naturalist*, 149(2), 336–351.