

Small Things, Big Consequences

Microbiological Perspectives on Biology

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Abstract Microbiology is a broad-ranging area of research that has developed out of 400 years of observation, analysis and theorizing about microscopic life forms. The study of microbes has not yet received a great deal of attention from philosophy of biology, but there are many reasons why it should. In this chapter, we outline the value of thinking philosophically about microbes and microbiology via a discussion of concepts of life, biological individuals and levels of selection. These discussions will show how taking a philosophical perspective on microbiological studies can enrich not only microbiology but also biology in general and its philosophy. We conclude by drawing out some of the implications of philosophical perspectives on microbiology for educational strategies in the teaching of biology.

1. Introduction

Microbes are the most numerous, diverse and ancient of the many life forms on our planet. They are also central to all life and its maintenance. The science of these organisms, microbiology, is the science of how microorganisms function, interact and evolve, in the context of causally influencing all other life forms. So far, microbiology has attracted barely any attention from philosophers of biology, and we outline the many reasons for remedying this state of affairs. We begin this chapter with a general argument for a philosophy of microbiology that recognizes the biological and evolutionary importance of the microbial world, and starts with a basic understanding of what microbes and microbiology are.

1.1. The entities studied by microbiology

'Microbe' is a general colloquial term that covers quite a range of microscopic biological phenomena. It includes all unicellular forms of life and commonly encompasses viruses, despite the fact that these entities have no cells of their own and have to use cellular organisms for reproduction. For this reason, even though they can be said to evolve, viruses are not usually thought of as living organisms (see Section 2 for debates on this topic). Unicellular microbes form three major groups, and these groups are now thought of as representative of the three major 'domains' of life: Bacteria, Archaea, Eukarya. Two of these microbial groups are bacteria and archaea, which together are called prokaryotes. Prokaryotes are unicellular life forms whose intracellular structure is organized without easily recognized compartmentalized functions, such as the nucleus (however, more and more compartments have been recognized in prokaryote cells, so they cannot be thought of as chemicals bounded by membranes). Bacteria and archaea used to be thought of only as bacteria, but fine-grained molecular and cell-biological work in the 1970s showed them to be very different physiologically and genetically. Their genomic content, cell walls, membranes, and replication, transcription and translation machinery all establish these two groups as very distinct life forms even if morphologically they are not obviously distinguished (Cavicchioli 2007).

The third group of unicellular life forms are found in the broad domain or superkingdom of eukaryotes, as are all multicellular organisms (as traditionally understood – cf. Section 3). Eukaryotes thus comprise all the unicellular and multicellular life forms that have the most well recognized compartmentalized processes in each cell. Eukaryotic microbes include protists, another colloquially named group, which are distinct from the other groups of eukaryotes (plants, animal and fungi), and are also distinguished from unicellular algae (microbes in the plant kingdom) and unicellular fungi (e.g., yeast).

Because 'microbe' means microscopic biological entity (versus 'macrobe'), informal discussions sometimes include very small multicellular organisms such as rotifers (mostly asexual animals found in aquatic environments). Not many microbiologists would do that, however (preferring the distinctions of 'microfauna' or 'meiofauna'), even though they and other biologists are perfectly willing to incorporate visible aggregations of single microbial cells such as filaments and moulds under that heading (it could be argued that organisms such as ourselves would then qualify as microbes, because we too are visible aggregations of single cells, organized in a particular way, as are filaments and other visible, organized aggregations of microorganisms). Although there are many troublesome issues in the nomenclature and taxonomy of microbes, our chapter will not focus on these but instead try to get at some of the deeper underlying issues of biological organization and its evolution.

1.2. Methods and perspectives in microbiology

Even a sketchy understanding of the development of microbiology as a science gives good reasons for philosophers to care about microbes. Microbiology has a long history, from the 1660s at least, which is when microscopy permitted close-up microbial observation and experiment. As well as accumulating diverse observations of miniature living things, the microscope provoked reflections on the very nature of microbial and other microscopic life (Hooke 1665; Leeuwenhoek 1694). These reflections led very naturally to profound questions about how life was generated, spontaneously or otherwise (Farley 1974). The more microscopic organisms were observed, the more pressing became questions about their specificity, stability and classifiability. The emergence of pure culture techniques in the late nineteenth century was a tremendous breakthrough for microbiology, since it enabled the stable identification of specific effects of different microbial taxa and thus their recognition as ‘species’ (Cohn 1875, in Brock 1961; Amsterdamska 1987; Bulloch 1938; Drews 2000; Gradmann 2000). Although in many respects, microbes were problem organisms for classification from Linnaeus’s time onwards (Ratcliff 2009), these problems did not stop proposals first in the nineteenth century and then in the mid-twentieth that microbes would make ideal model organisms for all biological study, and especially for genetic and biochemical analyses (e.g., Beijerinck 1900-1901; Theunissen 1996; Bateson 1907, in Summers 1991; Kluyver and Donker 1926, in Friedmann 2004; Demerec 1946; Lederberg 1987). This is ultimately how microbes became the platform for the great golden era of molecular biology in the mid-twentieth century, in which viruses and bacteria as well as some eukaryotic microbes became not just models of life but highly effective laboratory tools.

The centrality of microbes to molecular biology has been even more pronounced in the contemporary genomic era, in which first viruses and then prokaryotes had their whole genomes sequenced and analyzed. Now, with a molecular emphasis on systems, microbes are once again a major focus of general biology. This is not just because of the tractability of microbes, especially prokaryotes, but also because of the presumed unity of life that assumes common characteristics of life in all organisms, no matter how many cells constitute that life or how many compartments in any cell. While there are some important differences between microbial and multicellular life forms, especially in regard to evolutionary processes and patterns, as well as development (see Love, this volume), there is still a strong tendency in biology to emphasize shared properties as the basis of life and evolvability.

1.3. Why should philosophy of biology include microbiology?

We will take it for granted that philosophy of biology is concerned with life and the sciences that study it. Whatever the interests of any biologist or philosopher of biology, even if they have specific reasons to focus on the non-microbial aspects of animals, plants and fungi, microbes will be part of those specific biological phenomena and their environments. One reason for this claim is that most biodiversity on Earth is and always has been microbial, despite the fact that animals, fungi and plants are the most visible forms of life. The traditional unit of biodiversity is species, and although the concept of species is even more problematic in microbes than anywhere else (Ereshefsky 2010), microbiologists still use taxonomic units. Even after taking into account the many problems of sampling and scale, there are more microbial or even just prokaryotic taxa than there are multicellular taxa (Fierer and Lennon 2011).

On a strictly quantitative basis of entity counts, microbes outnumber all other life forms combined, even if the prolific viruses are not counted (Whitman et al. 1998; Suttle 2007). More than half the living biomass on the planet is prokaryotic (excluding the structural material supporting many plants), despite the much tinier size of prokaryote cells. Every environment on, in or around the planet is occupied by microbial life, whether we are examining the stratosphere or the deepest parts of the planet accessed by human technology (Nee 2004; Wainwright et al. 2004; Newman and Banfield 2002; Pedersen 2000). All cells host a variety of microbes, even prokaryote cells, which are themselves occupied by numerous viruses known as phages (with a few exceptions; see Willner et al. 2011).

But the most important form of biodiversity is metabolic. Microbes can do everything plants, animals and fungi do, and have many unique metabolic tricks up their sleeves. They can use organic and inorganic energy sources, respire aerobically or anaerobically, and fix their own or use already fixed carbon. Prokaryotes and other microbes can combine these metabolic strategies and switch from one to another; they can consort metabolically with other organisms and use or produce substrates from or for these metabolic partners (Madigan et al. 2008; Southam et al. 2007).

Microorganisms are not only producers of their own diversity but are fundamental to the maintenance of plant, animal and fungal biodiversity. The planetary chemistry of life is regulated by microbial metabolism interacting with the Earth's geochemistry (Falkowski et al. 2008; Newman and Banfield 2002; Dietrich et al. 2006). Most of the biogeochemical transformations necessary for life depend on interconnected metabolic pathways in microbes (Falkowski et al. 2008; Strom 2008). Carbon, oxygen and nitrogen cycles are essential for life on our planet, and microbes are major players in every one of these cycles and several others (Ingraham 2010; Kasting 2005; Newman and Banfield 2002). This is what we mean when we say that microbes form the basis of all fundamental life pro-

cesses and are thus the basis of all the biodiversity philosophers and biologists normally talk about.

Nor is this a recent development. Microbes have dominated all the past eras of the evolving Earth. From the origins of life until now, life has mostly been microbial. Whatever happens in the next great extinction event, many microbes are likely to survive it and continue evolving. If life is ever found anywhere else in the universe, it is most likely to be microbial (Gould 1994) or in the less probable case of multicellular life, to have arisen from microbes. It is well accepted that on our planet, microbes have been the evolutionary bases of all non-microbial life. Eukaryotic life arose from unicellular life; multicellular life began on the basis of eukaryotic microbes (Buss 1987; Bonner 1998; Maynard Smith and Szathmáry 1995; King 2004). All subsequent innovations, such as those of metazoan body plans and flowering plants, have their origins in a microbial context.

The dependence of multicellular life on unicellular life forms is ongoing in biology today. There is a vast array of symbioses that operate at every level of life. Symbiotic relationships, which may be mutualistic, commensal or parasitic, include endosymbioses (within cells), arrangements on the outside of cells (such as ‘dancing Yeti crabs’ that cultivate microorganisms as food by waving their furry claws over hydrothermal vents – see Thurber et al. 2011), and extensive partnerships between diverse groups of microorganisms (Moya et al. 2008). Every symbiosis involves microbes, even when the main symbionts are multicellular organisms. In humans, for example, our health, development and survival depend in a variety of ways on our microbial symbionts, many of which are parasites but which nevertheless interact intensively with us on both physiological and evolutionary timescales such that our ‘nature’ is shaped by our symbionts. Metagenomics, the molecular study of the interacting levels of biological organization constituting these collectives, is greatly advancing a more integrated view of biological entities and processes (O’Malley and Dupré 2009).

Our point is that the living world is saturated by microbes and their effects. They may be invisible individually, but collectively they constitute the greatest biological force on the planet. Because of this deep reliance and interdependence of all life on microbes, biologists and philosophers of biology have no choice but to consider microorganisms at least occasionally; far more if they are aiming at a deep embracing view of biology and the phenomena and processes of which it consists. We argue that using a microbiological perspective to analyse concepts central to biology—life, biological individuality and levels of selection—greatly enhances biological discourse by challenging the assumptions on which these concepts are based. Furthermore, demonstrating the connections between these notions reinforces the centrality of microbiological thinking to not only biology itself but also its philosophy and education.

2. Conceptions of life in classifying viruses as living things

Biology is the study of life and living things. The problem is that life itself is difficult to identify and define (see Cleland this volume), despite the intuitive difference between a giraffe and a rock. Some microbes challenge any common-sense notion of living and non-living. Indeed, whether a virus is alive has remained a puzzling question since their discovery over a century ago, and scientific evidence amassed since then has only complicated the matter. Even more dramatic but less obvious is the fact that the so-called ‘virus debate’ has complicated the very objective of defining life. The way scientists and philosophers engage in discussions about the living status of viruses underscores disparate ways of answering the question “What is life?”. Answers are given as either uncovering a natural kind or creating a useful heuristic for the categorization of biological entities. Because these two approaches to defining life are often conflated in arguments over the living status of viruses, approaching life from a microbiological perspective illuminates the nature of life debate in a unique way that disentangles natural kind and heuristic definitions.

2.1. *The living status of viruses*

Microscopic entities that seem to straddle the intersection of the living and the non-living, viruses challenge the common-sense distinction that seems so obvious when classifying much of microbial life. In the last several years, popular science magazines, professional virologists and philosophers have engaged in a debate that fuses questions about the living status of viruses and the definition of life in general (e.g. Forterre 2010; Moreira and Lopez-Garcia 2009; Owen 2008). In some ways, the rhetoric suggests that the debaters take life to be a genuine natural kind. In doing so, they follow in the spirit of definition of life debates in general, which often involve disputing proposed definitions by offering counter-examples (Cleland and Chyba 2002). The classification of living things, seen in this way, should be more than a heuristic tool for determining what sorts of entities biologists should study; rather, it marks out a real, unique group whose interactions with the world are profoundly different from their non-living counterparts. With a realist conception of life as a natural kind, the equally contentious auxiliary debate over the living status of viruses naturally follows. After all, if life is a natural grouping, viruses are either living or non-living, rather than living or non-living *by virtue* of a particular definition of life. Although virologists seem to want to draw the first conclusion, their arguments in fact only support the second.

Some definitions of life seem immediately amenable to viruses, such as that promulgated by Joyce (1995, p.140) and adopted by the USA’s National Aeronautics and Space Administration (NASA): ‘a self-sustained chemical system ca-

pable of undergoing Darwinian evolution'. Though a controversial objection, this definition may fail to capture early life if it did not undergo Darwinian evolution (Cleland and Chyba 2002). Further, definitions that merely require replication and evolution, without including a chemical system requirement, problematically count computer viruses among the living. Even if we are willing to accept this minimal definition, real viruses don't always fit the bill, argue Moreira and Lopez-Garcia (2009), for they are evolved *by* cells rather than evolved independently. Other definitions of life, such as Lwoff's (1967) widely accepted definition of life as cellular, exclude viruses with even greater certainty.

Yet, the recent discovery of a new group of viruses, disturbingly reminiscent of their living microbial counterparts, has challenged the classification of viruses as non-living and reignited debates about the definition of life. The so-called mimivirus, isolated in 1992 but only identified as a virus in 2003, shares critical features of living cellular organisms not previously known to be present in viruses (Claverie and Abergel 2009). Even the properties for which it was named—'mimi' being a reference to its mimicry of bacteria—illustrate the similarities between this virus and cellular life, and are why it took so long to be recognized as a virus rather than a parasitic bacterium (Raoult 2005). At 800 nanometres across it is much larger than most known viruses, and its 1.2 million base-pair genome encodes 1,260 genes, seven of which are common to all cellular life: eukaryotes, bacteria, and archaea. The mimivirus can even become 'ill' when infected by a smaller virus, thereby moving virologist Jean-Michel Claverie to exclaim that, 'The fact that it gets sick makes it more alive' (2008, p. 677, in Pearson 2008). In light of these findings, it seems that scientists must either concede that viruses are living organisms or commit to the view that non-living things can become infected.

2.2. Heuristic definitions of life

Whether or not these positions involve classifying viruses as living or non-living, both sides of the debate invoke scientific findings about viruses as evidence for their inclusion in either category. These arguments show that viruses are alive with respect to a given definition of life, with 'life' functioning as a heuristic that groups entities for study. That the definition of life is concurrently treated as a genuine natural kind, however, renders this ontological deflationism problematic. This is clear when virologists and others use scientific evidence to classify viruses as living or non-living while simultaneously recasting definitions of life to be either more inclusive or exclusive of them, thereby engaging in self-defeating debates. Forterre (2010), for example, uses the fact that viruses are alive with respect to a particular definition as evidence that viruses are alive *simpliciter* (belonging

to a natural kind). From this he reasons (circularly) that we should rewrite the definition of life to be more inclusive of viruses.

Raoult and Forterre (2008) call for the classification of viruses as living things on the basis of their similarities to organisms we currently consider living, thereby suggesting the viruses are alive with respect to our currently accepted definition of what a living organism is. Viruses should be considered living, they argue, because they possess the characteristics that other terrestrial life does: they are made of the same macromolecules and have co-evolved with the three domains of life. Hedge et al. (2009, in Ruiz-Saenz and Rodas 2010 p. 89) summarize similar evidence that suggests viruses are alive because they share features with organisms already considered alive: ‘viruses [...] follow Darwin’s theory of “survival of the fittest”, acquire mutations and evolve to sustain a new environment.’ That certain viruses also encode proteins shared by all three living domains and can become infected like their living counterparts—findings made possible because of the discovery of the mimivirus—supports the idea that viruses are in fact living entities.

Elsewhere, Forterre (2010) justifies his inclusion of viruses among the living by suggesting they are even consistent with what seems an impossibly exclusive definition of life: life as purely cellular. In his description, viruses normally form viral ‘factories’ in order to copy their genomes and produce virions that are dispersed and infect other hosts. Though often confused with the virus itself, according to Forterre, the virion is the part of the viral life cycle in which it is inactive outside of the cell in the form of a protective coat (capsid) encasing genetic material. Because mimivirus factories are nearly the size of the nucleus of their amoebic hosts, scientists have acknowledged that viral factories are more complex than once thought. Claverie (2010) has even proposed that the viral factory corresponds to the real organism, such that viruses virtually transform their cellular host into a viral factory. Once the cellular machinery has been co-opted into replicating viral DNA and producing virions, the cell is actually effectively a virus, Forterre (2010) contends. Even through the lens of the cellular definition of life, then, viruses are living things.

In counter-arguments that viruses are not genuine instances of life, life is also treated as a heuristic that usefully groups biological entities for study without identifying a natural kind. In their paper ‘Ten reasons to exclude viruses from the tree of life’, Moreira and Lopez-Garcia (2009, p. 307) immediately deflate the ontological status of the definition of life, claiming that whether viruses are alive is a matter of ‘inference and logic starting from any given definition of life.’ They proceed by outlining the ways in which the characteristics of viruses are not consistent with any potentially viable definitions of life—even those that rely on self-replication and evolution rather than metabolism. Viruses, they point out, do not evolve by themselves but rather rely on their cellular hosts to do so. In this sense, these microscopic entities are not merely non-living (i.e., are not part of a natural grouping of living things): they are non-living *in virtue* of particular conceptions of life. The upshot is that we might construct a definition of life that would be inclusive of viruses, but such a definition would likely be too generous in its inclu-

sivity, offering membership to things such as computer viruses that few would be comfortable to consider living. This liberalism in membership would undermine the usefulness of life as a heuristic that indicates what sorts of entities biologists should study.

2.3. Life as a natural kind

Although new scientific evidence about viruses has only suggested they are alive with respect to particular definitions of life, and counterarguments similarly claim the opposite, Forterre (2010) treats the definition of life as picking out a natural kind. After all, if it is enough that viruses are considered living in light of the cellular definition of life, Forterre's job is done. Raoult and Forterre (2008), however, continue to argue that the life-likeness of viruses is grounds for a new, more inclusive, definition of life. On their view, capsid-encoding organisms (viruses) complement the other three domains of life, which are collectively to be called 'ribosome-encoding organisms' (all cells contain ribosomes, which translate transcribed DNA (mRNA) into proteins). Fully defined, capsid-encoding organisms 'are composed of proteins and nucleic acids, self-assemble into a nucleocapsid and use a ribosome-encoding organism to complete its life cycle' (Raoult and Forterre 2008, p 314).

Forterre's argument is one example of a problematic rhetorical framework also exemplified in Benner's (2010) project of defining life ideally. On Benner's view, an ideal definition of life serves as a standard by which we may evaluate whether all potential organisms, including those we might encounter on distant planets, are living or non-living. In his words, the definition must be inclusive of everything that has features we 'value' in life. Such a definition relies on life as a heuristic: a convenient categorization to mark out groups of organisms with relevantly similar features. Our current definition is likely to be imperfect, Benner concedes, and will require updating as we encounter new entities that we would like to consider living but which do not fit our definition. As soon as we believe this new form of life is possible—and therefore worth investigating in the same way we do other living things—we will consider it living. The features that most researchers value, however, are generated by heuristic definitions of life; we think that cellular organisms are alive, for example, because of preconceived cellular definitions of life. Thus, those valuable features cannot be used as the basis on which to construct a definition that aims to pick out a natural kind.

Overall, attempts to classify the living status of viruses highlight a conflation of natural kind and heuristic approaches to articulating definitions of life. A microbiological perspective is therefore mandatory for disentangling ways of answering the question "What is life?". These issues of categorization and ontology are emphasized even more when we step away from the broad categorization of life to the entities that exhibit biological individuality.

3. What are biological individuals in light of microbiology?

Despite the fact that biological individuals (or ‘organisms’) are one of the primary units of study in the life sciences, surprisingly little attention has been given to explicating precisely what they are. This is probably because the identification of biological individuals is taken to be unproblematic (even given the well known problems of defining life). However, closer consideration of the facts—once again those pertaining to the microbial world—reveals that biological individuality is far from straightforward. In this section we show how microbiology has changed the way biological individuals are viewed.

For most people, animals are the most clear-cut biological individuals. It is certainly uncontroversial that human beings, dogs, birds and so on are organisms. Yet more than 90 percent of cells in any ‘human’ body are microbial (Savage 1977) and the same is true for all other animals. Many of these symbiotic microorganisms play a vital role in digestion, immune response and health in general. Does this mean that these beneficial symbionts are part of the animal body? One might think not because microbial cells are genetically, developmentally, and reproductively distinguishable from animal cells. However, matters get more complicated when the phenomenon of endosymbiosis is considered. It is now well established that mitochondria in eukaryotes, chloroplasts in plants, and perhaps other organelles (substructures within a cell which perform a specific function such as energy production) were originally bacteria that became incorporated into the larger cell (Sagan 1967; Margulis 1970; Archibald 2011; van der Giezen 2011). The emergence of eukaryotes, and of photosynthesizing eukaryotes, occurred when one prokaryotic organism assimilated another one by a form of cellular ingestion (‘endocytosis’, a major innovation in nutrient acquisition), and the fused cells became a single unit of inheritance and selection. Mitochondria and chloroplasts are the descendants of these engulfed prokaryotic individuals. Like bacteria, mitochondria reproduce by splitting into two, but this process is regulated in various ways by the host cell (Osteryoung and Nunnari 2003). Mitochondria also lack some of their original genome, which is instead located in the nucleus of the host cell. Because of this reliance on the host cell, mitochondria are not considered to be individual organisms (see section 4.3), although there is no scientific doubt that they once were. Nevertheless, this is clearly a matter of degree, as they still carry many of their own genes and reproduce semi-independently.

Discussion of symbionts and endosymbionts highlights a number of dimensions implicit in the notion of biological individuality. Some of those discussed by philosophers are very specific, and include germ-soma separation (reproduction specialist cells versus survival-and-growth cells), policing mechanisms (for punishing cheating and rewarding cooperation, as well as keeping foreign entities at bay).

spatial boundaries (demarcating the limits of the individual), co-dispersal (coupled reproduction and spread of the components of the individual) and being bearers of adaptations (Clarke 2010). Three more general dimensions have been discussed in detail by philosopher Jack Wilson (1999). The first of these is *genetic individuality*. In this dimension, organisms are distinguished from one another by their genetic makeup, with a biological individual being made up of more or less genetically identical cells. Genetic homogeneity is not enough, however, to distinguish parts of an individual from the whole, so a genetic individual needs to be genetically homogenous *and* genetically unique (Santelices 1999). The cells in a human heart, for example, are genetically homogenous but not unique because there are other genetically identical cells elsewhere in the same body. Another dimension of biological individuals is developmental. *Developmental individuals* are groups of cells that have developed from a single cell or small group of cells. Host organisms and their symbionts usually have different developmental trajectories despite the fact that host organisms often pass on symbionts to their offspring. The third feature of biological individuals identified by Wilson (1999) is functional integration. *Functional individuals* have parts that are strongly causally connected to one another but not with parts outside the individual. It is important to note that functional integration, just like developmental unity and genetic homogeneity, comes in degrees. There appears to be no sharp line demarcating any of these properties, thus making the identification of anything like natural kinds difficult.

For paradigmatic organisms, genetic, developmental and functional individuality appear at first glance to be neatly aligned. A typical animal is made up of functionally interdependent genetically unique and homogenous cells that develop from a single fertilized egg. Of course there are exceptions to this, such as identical (monozygotic) twins, which form a single genetic individual but two functional individuals, and either one or two developmental individuals depending on which starting point is selected (i.e., before or after the fertilized egg splits). The importance of considering microbiology does not simply derive from the fact that it furnishes us with more exceptions like this. Instead microbiology demonstrates that alignment of Wilson's three dimensions is the exception rather than the rule (Dupré and O'Malley 2009). In almost all cases, including paradigmatic biological individuals, the first two criteria are in tension with the third. This is because symbiotic communities, which are by definition genetically heterogeneous, are often functionally integrated wholes. Microbiological research has shown that biological individuality is more complicated than it might have seemed to be.

One of many examples of this is the glassy-winged sharpshooter (*Homalodisca vitripennis*), which is a kind of leafhopper that feeds solely on the sap of woody plants. Because this sap is low in nutrients, the sharpshooter depends on two bacteria, *Baumannia cicadellinicola* and *Sulcia muelleri*, to convert it into vitamins, amino acids and cofactors (Wu et al. 2006). The bacteria in turn rely on the host and one another for their survival, making all three highly co-dependent. Thus the bacteria function very much as parts of the sharpshooter's metabolic system despite being genetically and developmentally distinguishable. Such co-dependence

is the norm amongst related insects such as aphids, and allows them to exploit unclaimed ecological niches (Wu et al. 2006). Besides this metabolic role bacteria are also known to play an important role in invertebrate reproduction. Intracellular bacteria *Wolbachia* kill or feminize male organisms, such as *Drosophila*, induce parthenogenesis, and block successful mating between infected males and uninfected females (Werren et al. 2008). *Wolbachia* therefore function as part of the reproductive system of infected invertebrates. This kind of functional integration of symbiotic microorganisms with their hosts is also found in many other animals as well as plants (Barrow et al. 2008).

Microbiology also provides important insights into intra-specific relationships, or relationships between genetically related organisms. It is now widely believed that multicellular organisms evolved from highly cooperative groups of conspecific unicellular organisms (Buss 1987; Maynard Smith and Szathmáry 1995). This emphasis on cooperation is a common theme in much recent work on biological individuality (e.g., Folse and Roughgarden 2010; Michod and Roze 2001; Queller and Strassmann 2009). Research into microbial sociality is seen by many as vital for understanding how multicellular organisms evolved, and also how they function. In fact, many microorganisms are highly social, making them ideal candidates for studying the evolution of cooperation and individuality (Crespi 2001; Shapiro 1998; Velicer 2003). For instance, members of the ‘social’ slime mould, *Dictyostelium discoideum*, begin their lives as individual amoebae that aggregate together when food is scarce to form a slug that can move faster and traverse environments the individual amoeba cannot. Eventually they form a fruiting body consisting of a sterile stalk on top of which are fertile spores. The stalk is made up of dead amoebae, which give up their lives for the colony (Bonner 2009). Because these latter stages of the *Dictyostelium* life-cycle resemble a single organism in many ways and require a great deal of cooperation (often between genetically heterogeneous amoebae) it is an ideal case study.

This research on cooperation and sociality provides ways of investigating functional integration within an evolutionary framework. Instead of saying that parts of an organism are causally interdependent, the focus becomes the alignment of fitness of these parts and group-level adaptations (e.g. Folse and Roughgarden 2010; Strassmann and Queller 2010). The more interdependent the parts of an individual are, the more they will function as a single entity and share a common evolutionary fate. Placing biological individuals within a microbiological framework, therefore, is crucial for understanding both what living things are and how they evolve.

From a non-evolutionary perspective, there are several different kinds of equally important biological individuals, such as developmental, genetic, and functional individuals. However, from an evolutionary perspective (more specifically, a Darwinian perspective – see section 4), there is scope to argue that genetic and developmental individuality, as well as criteria such as germ-soma separation, policing mechanisms and spatial boundaries, are all secondary to cooperation or functional integration. That is, these other factors are all means by which high levels of cooperation are achieved and maintained rather than essential conditions

(Michod and Roze 2001; Queller and Strassmann 2009). They are adaptations for cooperation and functional integration.

For instance, genetic homogeneity in a group of cells can facilitate cooperation due to kin selection. An organism can pass on its genes either by reproducing itself or by increasing the chances of genetically similar organisms (i.e., kin) reproducing. Cooperative behaviour should thus be more likely amongst kin than amongst non-kin, all other things being equal. In turn, germ-soma separation and policing mechanisms can both be understood as mechanisms by which genetic homogeneity is maintained. As for spatial boundaries, Sterelny and Griffiths (1999) argue that the presence of a physical boundary isolates the individual cell or group of cells, promoting and maintaining functional integration.

From a Darwinian perspective, therefore, most of the characteristics associated with paradigmatic biological individuals are only contingent: our mistake has been to treat them as defining properties. Nonetheless, the presence of these characteristics on this view is often symptomatic of biological individuality. Do symbiotic groups show any such symptoms? In the case of microbe-macrobe symbioses it seems that they often do. Frank (1996) argues that host organisms often control symbiont reproduction by inducing a germ-soma separation in the symbiont. Microbes that live inside host cells (endosymbionts) share the cell wall as a spatial boundary, which reinforces their relationship with the host and encourages long-term interaction. And host immune systems act as policing mechanisms against free-riding or pathogenic microorganisms while tolerating commensalists and mutualists. These phenomena suggest to us that many microbe-macrobe symbioses, such as the glassy-winged sharpshooter and its endosymbionts, constitute biological individuals or at least crucial units of study.

Whatever position anyone might favour in this discussion, it is clear that advances in microbiology have drastically changed the way biological individuality is viewed, and will likely continue to do so. The genetic, developmental, and functional dimensions of biological individuality are not typically aligned as has traditionally been assumed. Furthermore, research into the relationships between these dimensions, involving particularly the study of microbe sociality, is at the forefront of both scientific and philosophical thought on this topic.

4. Can microbes help to understand and solve some of the issues in the debate about levels of selection?

Our discussion of biological individuality suggests that no real consensus about what an organism is can be achieved within a non-evolutionary framework. The situation is similar to the concept of species (e.g., Ereshefsky 2010). Each concept has several working definitions in different disciplines, without complete agreement on what constitutes a species or a biological individual. We proposed, however, that in the latter case, the situation might be clarified through a Darwinian

analysis acknowledging a microbiological perspective. Indeed, we have seen that new biological individuals can be formed during symbioses. Although these new (functional) individuals are not paradigmatic biological individuals (i.e., multicellular organisms), they nevertheless cannot be disqualified as viable biological individuals existing at a higher level than the symbionts on their own. Extending the notion of biological individuality to levels other than the organism level is precisely what is at stake in the levels of selection debate.¹

Without a microbiological perspective, this debate would probably have remained stuck at the same stage it had reached at the end of the 1960s, with the organism level being seen as the only level of selection ('individual selectionism'). A better understanding of microbial evolution revealed that what was thought about the primacy of the organism level was insufficient for a more general understanding of evolution by natural selection. A large body of empirical work on microbial evolution showed that group selection was an important force in evolution, contrary to what the individual selectionists of the late 1960s believed. More recent work on major transitions in individuality shows that what were thought to be 'true' individuals by individual selectionists in fact originated from groups of cells. This means that the argument for the individual organism as the sole level of selection is at least inadequate and at worst perniciously misleading. A microbiological perspective on evolution is therefore necessary for anyone who wants to appreciate the subtleties in the levels of selection debate.

4.1. The levels of selection debate in a nutshell

Prior to the 1960s, groups of multicellular organisms, such as mammals or birds, were usually uncritically considered to function harmoniously for the good of the group or the species. Under this view, which D.S. Wilson and E.O. Wilson (2007) label retrospectively 'naïve group selectionism', groups or species were the only true units of selection or 'Darwinian individuals'. However, this idea was forcefully debunked by Williams in his 1966 book *Adaptation and Natural Selection*. Although he recognized in principle the possibility of group selection, he stressed its theoretical implausibility in light of the models of that time, and the lack of empirical evidence for group selection as an important evolutionary force. Moreover at the time that Williams wrote his book, new theories were emerging as alternatives to group selection: inclusive fitness theory and the closely related kin selection

¹ For clarity's sake, we emphasize here that our claim about biological individuals existing at the group level is distinct from the claim made by Ghiselin (1974) and Hull (1976) that species are *individuals*. The individuals discussed in our paper are functional individuals, organisms, or units of selection, whereas the individuals Ghiselin and Hull care about are merely spatiotemporally localized entities (Wilson and Sober 1989).

theory (Hamilton 1964), evolutionary game theory (Axelrod 1984; Maynard Smith 1982) and selfish gene theory, also known as the ‘gene’s eye view’ (Dawkins 1976; 1982).

By the beginning of the 1970s, the concept of group selection had been renounced by all respectable evolutionary biologists. The most popular argument for the organism as the primary level of selection was that selection at the level of the group would always be disrupted by selection at lower levels. For example, within a group of altruistic individuals sacrificing their interests by helping others, a selfish mutant will have higher fitness than an altruist. This type of reasoning leads most evolutionary biologists even today to think of the organismal individual as the sole significant Darwinian individual. Wilson and Wilson (2007) argue convincingly that this individualistic evolutionary perspective, which always refers to the paradigmatic biological perspective of multicellular organisms, is misguided. They advocate ‘neo-group-selectionism’. The difference between naïve group selectionism and neo-group-selectionism is that the latter is placed within a general theory of multilevel selection, which recognizes that all the different levels of organization can be relevant levels of selection, with Darwinian individuals nested within one another.

Wilson and Wilson propose three main reasons in favour of neo-group-selectionism and for each reason a microbiological perspective is essential. The first is that new theoretical models, especially agent-based models (unavailable in the 1960s) clearly and plausibly demonstrate how group selection can occur. Furthermore, some models of virulence show that under realistic assumptions virulence is expected to decrease over time (Bull 1994). The decrease of virulence can hardly be explained *without* a multilevel perspective. Although it is in the interests of the individual pathogen to use as many resources as possible in order to spread (which would kill its host), it is not in the interest of the group of host-pathogens, since the population of hosts is not infinite and killing all of them would result in the conjoint extinction of the pathogens. Decreasing the level of the virulence is hence adaptive at the group level.

Second, many empirical studies show that group selection is an important evolutionary force, or at least that evolution can be interpreted legitimately from a group-selectionist perspective. Much of the best evidence for this claim comes from microbial evolution. A recent exemplar is Rainey and Rainey’s work (2003) on the ‘wrinkly spreader (WS)’ strain (sub-species) of the bacterium, *Pseudomonas fluorescens*. This strain produces a mat on the surface of a liquid medium. Although this mat is costly to produce and strains of *P. fluorescens* that do not contribute to its production (cheaters) have the highest fitness within groups of mixed strains, the WS strain is maintained in the overall population by inter-group selection: groups in which the WS strain is present do better than others. More recently, Rainey and Kerr (2010) have argued that WS cheaters form the germline of the proto-multicellular organisms constituted by the mat. This represents a new microbiological hypothesis about the origin of multicellular organisms, which (as we will see in 4.3) is an important topic within the levels of selection debate. An-

other microbiological example of group selection comes from Kerr et al. (2006), who present an experimental setting of a metapopulation (a population of populations) in which the T4 phage can adopt two strategies for killing its host, the bacterium *Escherichia coli*, depending on the dynamics of migration between the different populations. If the migration is unrestricted, ‘rapacious’ strains of phages out-compete ‘prudent’ strains, which kill the host more slowly and thus allow bacterial populations to reproduce more before being infected. However, when migration is limited and matched by actual migration rates between populations, the more prudent strains out-compete the rapacious ones. This study is an empirical confirmation of the models of virulence discussed above.

The third piece of evidence that Wilson and Wilson cite in support of neo-group-selectionism is that the three main theories presented as alternatives to group selection are in fact perfectly consistent with it. Inclusive fitness theory is nowadays seen as formally equivalent to group selection (Okasha 2006; Wilson and Wilson 2007; West et al. 2007). The two theories are fully translatable, and this is recognized by many neo-group selectionists *and* individual selectionists (however, cf. van Veelen et al. 2011; Nowak et al 2010). In addition, the models used in evolutionary game theory implicitly invoke groups, because when n individuals interact they form a group of size n , and during these interactions different strategies can be selected in different groups. Although microbiology is not directly implicated in these theoretical considerations, the development of these two theories would certainly have had a different structure if they had taken microbial groups into account. The group-level evolution of microbes can readily be studied due to their size and rapid generation time (unlike animals, for example).

Finally, say Wilson and Wilson, selfish gene theory is neither an alternative theory to group selection, nor a theory demonstrating the prevalence of individual level selection since it confuses mere ‘bookkeeping’ with causality (Gould and Lloyd 1999; Okasha 2006). In other words, while it is true that any evolutionary process will have an outcome at the level of genes, it does not follow that genes causally explain this outcome or that the individual organism level should be privileged (Okasha 2006). This is especially true when biological individuality is itself a blurry concept, and becomes even more apparent when we examine major evolutionary transitions in such individuality.

4.3. Microbiology at the heart of the major transitions in individuality

The levels of selection debate went through a dramatic shift under the influence of Buss and his 1987 book *The Evolution of Individuality*, and Maynard-Smith and Szathmáry and their *The Major Transitions in Evolution* (1995). These accounts show that modern multicellular organisms are the evolutionary product of cooperation amongst groups of microorganisms in which conflicts had been resolved

(although see Clarke 2011 for a different account on non-resolution of conflicts in plants). Under this view, individual level selection could be understood as a form of group selection: selection between groups of cells. An individual selectionist could argue that whether we call these cells ‘an individual group of cells’ or ‘an individual’ is purely a semantic matter, and that their core argument is that this level is the only important one in evolution. However, this position misses a crucial aspect of the transition from uni- to multicellular organisms, as well as other evolutionary transitions such as the origins of life. The fact that multicellular organisms arose from unicellular organisms means necessarily that group selection was the most important force in evolution during this transition. It was so important that it created what we call today the ‘individual level’—a level that did not exist before.

This transition into individuality makes it clear that an exclusively individualist position on selection is untenable. Arguing against group selection as an important force in evolution nowadays (which is mostly what the levels of selection debate is about), commits the individual selectionist to hold that evolution is only about relatively recent multicellular organisms. Moreover, some extant organisms seriously challenge the very concept of multicellularity. This is the case for the slime mould already mentioned, *Dictyostelium*, which is part of the time a solitary amoeba and part of the time a collection of amoebae that reproduces like a paradigmatic multicellular organism (i.e. some of the amoebae become ‘somatic cells’ while others become ‘germ cells’ and form a fruiting body; for more details see Bonner 2009). Myxobacteria are a similar example in the prokaryotic world (Shimkets 1990).

Arguing against group selection also commits individual selectionists to the denial of questions about origins of life as an important topic in evolutionary biology. While it is legitimate for a science not to be interested in the question of origins, it is at least a paradoxical decision in a discipline called ‘evolutionary biology’, especially when ‘origin’ means the origin of evolutionary innovations. As Buss (1987, p. 20) notes, individuality is itself an innovation that needs to be explained. An organismal perspective remains deaf to the question of the origin of multicellular organisms from groups of microorganisms. Finally, arguing against group selection and, more generally, multiple levels of selection commits one to ignoring the creation of new individuals from two or more genetically distinct actors. We have already mentioned endosymbiosis theory (Margulis 1970), widely accepted nowadays, which proposes that the eukaryotic cell is a chimera constituted by what were initially distinct individuals. From their endosymbiosis onwards, natural selection acted on this group of organisms as a whole, causing not only the evolutionary future of these organisms to be intertwined but also their increasing biological dependence.

Overall, we suggest that microbiology reveals that a commitment to a single level of selection, namely the traditional organismal level, is too narrow both in light of genuine examples of group selection (many of which are microbial), and because of the problematization of the very concept of the individual. Microbiolo-

gy is clearly playing a crucial role in the levels of selection debate, which has been a central topic of discussion in evolutionary biology for several decades. Evolutionary analyses of microbial systems have allowed biologists to test new hypotheses, given them new conceptual tools, and helped them clarify the questions at stake in the debate.

5. Philosophy of microbiology and biology education

Each of our three case studies illustrates the contributions of a microbiological perspective to biology and philosophy of biology debates. While our discussions above about defining life, biological individuality and levels of selection speak for themselves, we want to emphasize as our conclusion that there are numerous ways in which a philosophical understanding of microbiology can contribute to education in biology. The first reason is an obvious one: additional information about microbes can be incorporated into a broader biological understanding. This would not necessarily be philosophical, but the philosophical issues we have spelled out could act as an incentive for microbial biologists to learn a little about microbiology. From a more explicitly philosophical perspective, debates, problems, and unresolved issues in macrobiology can be critically assessed in light of microbiology. Most generally of all, philosophy of biology, by including microbiology, could widen the scope of biology and conceptions of how science is practised.

While microbiology contributes to major philosophical questions that are relevant to all philosophical and historical efforts to understand biology, the discipline of microbiology itself can benefit from an appreciation of its underlying philosophical dimensions. Even though microbiologists have long advocated a broad integrative view of microbiology education (Handelsman 2002), its integration can extend to biology more generally. It is not uncommon for biologists to ignore microbiology, especially its ecological and evolutionary aspects, and our cases above show clearly why this would be a problem.

Not only does the philosophy of microbiology generate a more inclusive representation of life, but it also tests many standard biological assumptions about the study of life. One of them is in regard to the debate about reductionism, which has been an important topic in philosophy of biology, but is now shifting towards closer scrutiny of multilevel integrative explanations (Brigandt and Love 2012; Mitchell 2003). We have shown from a microbiological point of view that methodologically, even if the macroorganism is the primary object of study, it must be understood both in a microbial context *and* in terms of a microbial decomposition. If we want to understand global warming, for example, microbes have to be brought into a broad-brush picture of atmospheric gases, temperature trends and ocean acidification (Singh et al. 2010; Zehr et al. 2011). But at the same time, individual genomes, pathways, and organismal interactions have to be understood at the level of the single cell in order to obtain more precise information and capaci-

ties for intervention. Major social health problems, such as obesity, can only be understood when the effects of microbial communities on the generation of adipose tissues in traditional multicellular organisms (such as humans and rodents) are comprehended evolutionarily and ontogenetically (O’Malley and Stotz 2011). Being able to slide up and down ecological, evolutionary and organismal scales via microbial analysis is something we think our sections above show clearly. As a consequence, traditional concerns about reductionism, whether scientific or philosophical, become much less pressing. The epistemological issue that is brought to the forefront by microbiological analysis is therefore not so much reductionism as the integration and importance of multiple levels of analysis.

Philosophy of microbiology also has very important ontological questions to discuss that are highly relevant to all of biology. In this chapter, we have shown through our examination of life, biological individuality and levels of selection how the macrobe-microbe distinction is not ontologically meaningful, even though there can be many good practical reasons to focus on either microbial or non-microbial life. All organisms, no matter their size, are biological individuals of some sort, and yet it is primarily by including microbes in any discussion of the ontology of such individuality that the deep issues in such ontological attributions become clear. The same is true of the Darwinian individual, which we have shown here to be problematized by microbiology not just in a critical but a highly constructive way: once the traditional organism is not assumed as the focus of analysis, there is room to develop a much better understanding of group and multilevel selection. The modern synthesis of evolution, while it has undeniably revolutionized evolutionary biology, is largely the result of an a-microbial picture of the world. Because of the exclusion of microbial processes and patterns (including several we have not discussed here, such as how microbes share genetic resources), traditional forms of evolutionary theory cannot encompass major evolutionary questions, including those about the origins of life and major evolutionary transitions.

For many major philosophy of science discussions, such as those about natural kinds, pluralism and multilevel explanation, philosophy of microbiology has important contributions to make and can be part of a broader agenda of introducing philosophy of science to biology students. This is not for imperialistic disciplinary reasons, but for the simple reason that biologists need to draw out the most profound implications of the science they do. This does not always require discussion of microbes, of course, but it does mean that broad, abstract claims about ‘living things’, biodiversity and evolution should be examined in light of microbial life as well as in reference to visible life forms. We have only indicated the depth and scope of these issues in regard to a limited set of examples (life, biological individuality, levels of selection), and there is a great deal of work that could be taken up by anyone stimulated by these illustrations.

Overall, we see the philosophy of microbiology working as a corrective to a tradition of *not* thinking about microbes, whether that is in philosophy or biology. Thinking ‘microbiologically’ has provided valuable new insights into many tradi-

tional biology debates, whether over the definition of life, the concept of an individual or levels of selection. On top of this, a philosophically motivated microbiology education is vital for ensuring that the same shallow assumptions do not continue to be guiding principles. Instead of revisiting existing debates from a microbiological perspective (as we have done here), we are optimistic that future developments in biology can start from a more inclusive conception of biology. Biologists may sometimes find it useful to emphasize differences between microbial and macrobial life, but whatever is going on in the biological world, there is no way it can be totally abstracted from a microbial context, and any general biological claim needs testing against the microbial world.

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