SIZE DOESN'T MATTER:

TOWARDS A MORE INCLUSIVE PHILOSOPHY OF BIOLOGY

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Abstract

Philosophers of biology, along with everyone else, generally perceive life to fall into two broad categories, the microbes and macrobes, and then pay most of their attention to the latter. 'Macrobe' is the word we propose for larger life forms, and we use it as part of an argument for microbial equality. We suggest that taking more notice of microbes – the dominant life form on the planet, both now and throughout evolutionary history – will transform some of the philosophy of biology's standard ideas on ontology, evolution, taxonomy and biodiversity. Using insights from the emerging microbiological discipline of metagenomics breaks down conventional associations of one genome with one organism, and suggests the metagenome as a community resource both over evolutionary time (because of horizontal gene transfer) and for current function. An emphasis on community function allows an exploration of multicellularity through research on microbes' communal capacities for cooperation and communication. This research programme brings new perspectives to the levels of selection debate and discussions of the evolution of multicellularity, and as well as to neo-Darwinian understandings of evolutionary mechanisms. A closer appreciation of classification issues in microbiology mandates a much broader and more diverse philosophy of systematics, and offers a new dimension to the philosophy of biodiversity. Incorporating microbial insights into the philosophy of biology will mean some old habits have to be

abandoned, but will benefit the discipline by adding greater scope and depth to its investigations.

Keywords

Microbes, macrobes, microbiology, metagenome, metagenomics, ontology, evolution, multicellularity, taxonomy, biodiversity

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Introduction: Microbes and Macrobes

The distinction between micro- and macro-organisms is one of the most widely assumed in thinking about life forms. While we have two words for the first group – microorganisms or microbes – there is none in common use for macroorganisms. We propose to fill this gap with the word 'macrobe'.[1] The contrast between microbes and macrobes is very close to that between multi-celled and single-celled organisms. Microbes are also defined by features such as invisibility and a perceived lack of morphological and cellular sophistication; macrobes by a positive account of those features. But regardless of choice of defining features, neither of these categories would normally be attributed much biological coherence. Microbes comprise two of the three main currently recognised biological superkingdoms, Bacteria and Archaea, as well as the single-celled eukaryotic protists. Macrobes comprise the remainder of the Eukarya, the kingdoms Animalia (including the Metazoa), the Fungi and the Plantae.[2] The distinction between the two groups is not entirely sharp: various social single-celled organisms, both prokaryotic and eukaryotic, such as the myxobacteria and cellular slime moulds, have claims to multicellularity. We frame our argument round this distinction for two reasons, however. First, the macrobes are at least as coherent a group as the microbes, so it is worth reflecting on why the latter seems so much more natural a

concept than the former. But second, and this is the main thesis of this paper, we believe that an indefensible focus on macrobes has distorted several basic aspects of our philosophical view of the biological world.

The tendency of philosophers to overlook microbes is particularly remarkable in view of the fact that microorganisms dominate life on this planet, whether they are considered from an evolutionary or an ahistorical perspective. Evolutionarily, the first three billion years of life on the planet was primarily microbial, with the Cambrian explosion of modern multicellular metazoan body forms beginning only about 545 million years ago (Conway Morris 2003; Carroll 2001).[3] Microorganisms are deeply implicated in the geochemical development of the planet, from the formation of ore deposits to the biologically dramatic creation and maintenance of the oxic atmosphere on which macrobes depend (Kasting and Siefert 2002; Newman and Banfield 2002). Functionally, microbes have vastly greater metabolic and environmental diversity than macrobes, and can thrive in conditions that are intolerable for most plants and animals. Microbial species diversity is only estimated but it exceeds all other life forms, as do estimates of their global cell numbers.[4]

Given their fundamental and undisputed importance for all life – including human life – it is curious that the philosophy of biology has largely ignored microorganisms and microbiology. Whether talking about evolution or biodiversity, the philosophy of biology has focused almost exclusively on multicellular life.[5] Decades of heated philosophical discussion about systematics and concepts of species have either not noticed the

microbial world or found it convenient to dismiss it. Although it might be an interesting exercise to work out why this has happened, we are more interested in arguing for an end to such biological blindness. What we seek to do, therefore, is show the radical revisions new understandings of microbes force upon some long-established ways of thinking in the philosophy of biology.

The three areas we will focus on are traditional concerns for philosophers of biology: ontology, evolution, and taxonomy (including biodiversity). We will show what is missed by a macrobe-dominated philosophy of biology and how microbiological knowledge challenges some currently limited ways of thinking about life forms and processes. Although there are many streams of microbiology we could follow, here we will focus on molecular and evolutionary microbiology (sometimes called comparative evolutionary genomics) and a postgenomic field emerging out of it called metagenomics. We will start with an outline of the disciplinary background and current state of metagenomics, followed by a discussion of how the new field potentially transforms conventional ontologies of genomes and organisms, as well as philosophical understandings of evolution and taxonomy that are based on macrobes.

Metagenomics

Background: microbes, microbiology, systematics and genomics

In general, anything too small to be seen without a microscope is called a 'microbe' or 'microorganism'.[6] The category includes all prokaryotes (bacteria, and archaea), all unicellular protists (protozoa, algae and fungi) and viruses. Most are single cells, though some are cell clusters and viruses have no cells. We will focus on bacteria and archaea in this paper, though many fascinating stories and philosophical complications could also be drawn from viruses and protozoa (e.g.: Nanney 1999; Corliss 1999; Sapp 1987). Bacteria and archaea are distinguished from each other by important differences in cell wall chemistry, metabolic pathways, and transcriptional and translational machinery (Woese and Fox 1977; Bell and Jackson 1998; Brown 2001).

Although microbiology may have begun with the invention of the microscope, its dreams of a comprehensive understanding of biodiversity and evolutionary history only began to approach realization with the equally revolutionary tools of protein and DNA sequencing. This revolution was initiated by Carl Woese and his colleagues as an implementation of Zuckerkandl and Pauling's methodological outline of how to use molecules as fossils or documents of the evolutionary history of organisms (1963; 1965). Woese's discovery of the archaea dramatically transformed the basic classificatory framework from two fundamental domains of life (prokaryotes and eukaryotes[7]) to three, and cast new light on the origins and subsequent differentiation of biological lineages (Woese and Fox 1977; Fox et al. 1980). Although disputed by many taxonomists – especially those outside microbiology (e.g.: Mayr, 1998[8]) – Woese's work made more sense of molecular data and appeared finally to enable a 'natural' phylogenetic classification of bacteria instead of the prevailing phenetic approaches

used (sometimes reluctantly) as defaults (Olsen et al. 1986; Woese 1987; Woese et al. 1990; Young 2001).

Early cumbersome methods and limited sequences were rapidly overwhelmed by high-throughput whole-genome sequencing methods. The first microbial genome sequenced was that of *Haemophilus influenzae* in 1995 (Fleischmann et al. 1995), followed quickly by the smallest bacterial genome then known – *Mycoplasma genitalium* (Fraser et al. 1995) – and then the archaeal genome of *Methanococcus jannaschii* (Bult et al. 1996). There are now more than 230 whole prokaryote genomes sequenced (with 370 in the pipeline, and over 1500 virus genome sequences) – more than 12 times the number of eukaryote genomes available (www.ncbi.nlm.nih.gov/genomes). The comparative work done with these sequences has been enormous and has enabled an increasingly complex understanding of gene function and evolution (Fraser et al. 2000; Brown 2001; Doolittle 1998).

While comparative genomic studies confirmed the distinctiveness of the archaea, they also complicated the simpler stories told by popular single-gene phylogenetic markers such as the 16S ribosomal gene. More knowledge about genome composition revealed the complex histories of gene exchange and genomic mosaicism inherent in microbial evolution (Doolittle et al. 2002; Koonin et al. 2001; Lawrence and Hendrickson 2003; O'Malley and Boucher 2005). Partly in the hope that more data on more taxa would produce greater phylogenetic clarity, genomic analysis was extended beyond laboratory cultures of microorganisms to DNA extracted directly from natural environments (Stahl

et al. 1984; Olsen et al. 1986; Amann et al. 1995). This strategy sought to overcome the highly biased 'culture dependent' understanding of microbial characteristics and diversity that was an artefact of the technical difficulties of cultivating most prokaryotes (perhaps as many as 99% of them). While this move out of the laboratory vastly expanded the scope of the data collected as well as understandings of biodiversity and evolution (Pace 1997), the continued focus on particular genes as phylogenetic markers still gave limited assessments of diversity (Dykhuizen, 1998; Schloss and Handelsman 2004) and did not provide much information about the physiological or ecological importance of the organisms (Brune and Riedrich 2000; Rodríguez-Valera 2002; DeLong and Pace 2001; Staley and Gosink 1999).

Metagenomics

Metagenomics, or the application of genomic analysis to community genomes, could be considered the next revolutionary expansion of molecular microbiology in that it merges environmental scope across taxa with whole-genome breadth and promises to overcome the limitations inherent in studies based on either isolated genes or isolated organisms. Instead of generating molecular catalogues based on single genes, metagenomics aims to provide genome-oriented functional analyses of entire communities of complexly interacting organisms in diverse ecological contexts. The term 'metagenome' was first defined as the collective genome of the total microbiota of a specific environment by Jo Handelsman and colleagues in 1998 (Handelsman, et al.

1998) and ‘metagenomics’ is now applied retrospectively to some earlier studies that preceded the coining of the label (e.g.: Stein et al. 1996).

Currently, the study of metagenomes consists of cloning, screening and sequencing large quantities of environmental DNA (Handelsman et al. 1998; Béjà 2004; Rodríguez-Valera 2004; DeLong 2004a; Rondon et al. 2000; Riesenfeld et al. 2004). Sampled environments include ocean sediments (Breitbart et al. 2004), the human gut (Breitbart et al. 2003), the human oral cavity (Diaz-Torres et al. 2003) and drinking-water valves (Schmeisser et al. 2003). The most comprehensive metagenomic studies have shotgun-sequenced all the DNA in an environmental sample – both from environments with low species densities (Tyson et al. 2004) as well as from considerably more complex oceanic communities (Venter et al. 2004). Venter’s catalogue of sub-surface prokaryotes in the Sargasso Sea has generated the largest genomic dataset for any community and given rise to an expanded notion of ‘megagenome’ (Handelsman 2004). However, the full metagenome sequence of the most complex and diverse communities (especially in soils) is still beyond the reach of current technologies.

These studies are not only discovering new genes and strains of prokaryotes and viruses, but also revealing wholly unanticipated functions and mechanisms[9] such as photobiology in oceanic bacteria. Genes for light-driven energy production (proteorhodopsin genes) were well known in halophilic or salt-loving archaea but never before suspected in oceanic bacteria until discovered via metagenomic analysis (DeLong 2005; Béja et al. 2000). Another unexpected use of metagenomics was to

overcome the technical challenges of sequencing ancient DNA – in this case from the Pleistocene cave bear, *Ursus spelaeus*. By sequencing all the DNA in the sample, which included microbial, fungi, plant and animal contaminants, then comparing the metasequence against modern dog and bear sequences, it was possible to distinguish the cave bear DNA (Noonan et al. 2005). In this case, the interest was not the metagenome itself but an individual genome. Nevertheless, the viability of ancient DNA sequencing via 'palaeometagenomics' now seems established and there is talk of a 'Neanderthal Metagenome Project' (Rubin, in Pennisi 2005).

At present, metagenomics is in an intensive discovery phase not unlike that of early single-organism genomics. It is more concerned with constructing inventories of environmental sequence and gene products than able to give extensive insight into ecosystem function and physiology (Schloss and Handelsman 2003; Chen and Pachter 2005). The two most pressing epistemological issues in this early phase of metagenomics are straightforwardly methodological. The first is to recognize and overcome the methodological biases that limit or contaminate the detection of diversity (Riesenfeld et al. 2004; Rodríguez-Valera 2004; Streit and Schmitz 2004; Liles et al. 2003; Béjà 2004; Torsvik and Øvreås 2002; Wellington et al. 2003). This concern builds on the earlier recognitions of the limitations of culture-dependent and 16S cataloguing approaches. Because samples (rather than complete inventories) will always be the only practical means of approaching this diversity, dealing with sampling bias will always be an issue (Hughes et al. 2001).

The second methodological issue is the extension of existing tools for individual genome and proteome analysis so that not only can individual genomes be reconstructed from the metagenome data (thus according it more reliability) but also the complex functions of microbial communities in their environment can be analysed (DeLong 2005; Ram et al. 2005; Handelsman 2004; 2005; Wellington et al. 2003; Torsvik and Øvreås 2002; Sebat et al. 2003). Comparative metagenomics, or the comparison of the genomic diversity and activity of different microbial communities, has already begun (Tringe et al. 2005) and adds further analytic depth to metagenomics. The fact that genomic tools are already available for exploitation should enable metagenomics to develop quickly, but that development will also require the tighter integration of microbial ecology, population genomics, phylogeny and biogeochemistry (DeLong 2005; Rodríguez-Valera 2002).

The next phase of metagenomics is ambitiously forecast to be 'microbial systems science' in which microbial ecosystems are analysed by interdisciplinary teams as 'complex biological networks across multiple hierarchical levels' (DeLong 2002a; 2004a; Rodríguez-Valera 2004; Buckley 2004a). Although there is so far limited discussion of what this would involve, it is clear that a 'systems ecological' perspective would meld a molecular approach with the synthesizing perspective of ecosystems and thereby extend the current systems biology focus on intracellular interactions (DeLong 2005). It is this 'system' perspective, which takes networks of biological activity in ecological settings as its object, that has some of the most interesting implications for the

philosophy of biology and its traditional considerations about the units of fundamental ontological importance.

1. Ontology

The central ontological categories for traditional philosophy of biology have been the individual organism and the lineage, the latter sometimes extended to include the more controversial notion of species as individuals (Hull 1987b). Populations, whether sexually or asexually reproducing, have been conceived of as constructed out of individuals. Individual microbes have an unproblematic status in microbiology as well, but the notion of community (along with consortium or assemblage) has also deeply informed the discipline's theory and research. Metagenomics, often called 'community genomics', takes this perspective an ontological step further by shifting the scientific focus from individual genomes and organisms to a genetic commons and its dynamic use (Allen and Banfield 2005).

First of all, the metagenome itself is conceived of not just as a collection of individual genomes but as the genomic resource of a whole community both over evolutionary time and for current function: the whole is more than just the sum of its parts. Evolutionarily, the metagenome is recognized as the product of collective adaptation, dependent on the continuous possibility of gene exchange by a variety of mechanisms. Far from adaptation requiring the genetic isolation of lineages, as conceived in standard

evolutionary theory, the transfer of genetic material between and within lineages made possible by these mechanisms is what enables communities to adapt rapidly to changing environments. This capacity for resource exchange has been described as a distributed genome or a genetic free market (Sonea and Mathieu 2001) – a global resource too big for single cells but accessible when populations find ecological reasons to acquire DNA for new functions. A strong interpretation of gene transfer means that individual genomes are ephemeral entities fleetingly maintained 'by the vagaries of selection and chance', and taxa are only an 'epiphenomenon of differential barriers' (environmental, geographical and biological) to lateral gene transfer (Charlebois et al. 2003). Although some microbiologists would prefer to restrict the metagenome to a clade-specific metagenome or 'pan-genome' (the collection of genes shared by a species) (Lawrence and Hendrickson 2005), such a characterization is made on the basis of a stable core for a species genome – an assumption that does not fully capture the ontological implications of gene transfer, especially when instances of 'core' transfer are considered.

Even if the most radical conceptual implications of gene exchange are set aside, the analysis of how metagenomes function for communities existing in current environments is still ontologically revealing. Microbial communities are thought of not merely as groups of organisms in close proximity but as highly organized entities that have to be understood in terms of complexly interacting functions. Individuals are not ignored but are understood on the basis of their functional role in the community (Allen and Banfield 2005). A simple example of this approach in action is Tringe and colleagues' (2005)

metagenomic analysis of soil and whale carcass samples. The authors argue that proteins encoded by the community rather than the organisms producing them were more important for true functional and environmental insight. Consequently, the difficulties of individual genome reconstruction from metagenome sequence matter much less than they would from a single-genome perspective. Some microbiologists counsel against such a move, arguing that metagenomes should not be treated as a single entity but as thousands of genomes belonging to single cells: these are 'the critical units of organization in microbial communities and they should not be dissolved' (Buckley 2004b). Despite such qualms, metagenomics is indisputably characterized by its community orientation – a legacy bequeathed by both microbiology and ecology – that will always emphasize the collective over the individual and conveniently coincides with the current technological limitations on reconstructing individual genomes from metagenome data.

Metagenomics does not end at the strictly genomic level of analysis, of course, but inevitably moves into an analysis of the multilevel interactions between metagenomes, metaproteomes and metametabolomes, assemblages of organisms and their environments. The sum of these levels constitutes what are tentatively called 'metaorganisms' because of the clear parallels between the integrated functioning of microbial communities in ecosystems and the collaborative working of the tissues and organs of multicellular organisms (Rodríguez-Valera 2004; Cases and de Lorenzo 2002). Although more conservative readings of such claims may see them as simply metaphors for large-scale interactions between individual units (genomes and

organisms), the systems aims of metagenomics appear to override this interpretation – especially when considered in the light of a considerable literature that hypothesizes bacterial populations as multicellular entities.

Numerous studies of bacteria and other microbes show how scientifically helpful it is to conceptualize populations and communities as multicellular organisms (Shapiro 1998; Kolenbrander 2000). By working together as functional units, bacteria can effect a coordinated division of labour into zones of differentiating cell types that enable them access to a greater variety of energy sources, habitats, protection and other collective survival strategies[10] (Crespi 2000; Shapiro and Dworkin 1997). All these are functions individual bacteria are unable to accomplish, and, in fact, are often achieved at the expense of 'altruistic' individual microorganisms. Many of these strategies have been observed and experimented on in single-taxon populations, but highly co-ordinated forms of cooperation are also found in mixed (multi-taxa) consortia of bacteria. These range from carrying out coordinated cascades of metabolic processes to the regulation of host-parasite interactions (Shapiro 1998; Crespi 2001; Kolenbrander 2000). For all of these coordinated activities, signalling and regulatory mechanisms are essential. One form these take is quorum sensing or cell-to-cell communication between bacteria at high cell densities. The process assesses population density and responds to it by regulating gene expression that governs behaviours from virulence production to the production of bioluminescence (Dunny and Winans 1999; Miller and Bassler 2001; Henke and Bassler 2004). Again, such communication occurs not only within same-species populations but also between species in communities [11] (Federle and Bassler

2003). Cheater controls are obvious objects of investigation to understand the fine-tuning of cooperation in prokaryote communities[12] (Velicer 2003; Travisano and Velicer 2004).

The intriguing ontological hypothesis that metagenomics suggests is that communities are self-organizing entities that operate as functional units and are more than simple aggregations of individuals (Kolenbrander 2000; Ben-Jacob et al. 2000; Andrews 1998). This, in turn, suggests that rather than see macrobes as a 'higher' level of biological organization, we should view macrobes and microbial communities as constituting alternative strategies for coordinating the activities of multiple differentiated cells. Exploration of the differentiation of function within microbial communities (or metaorganisms) could prove a major source of insight into the nature of multicellularity and its evolutionary origins. In parallel with current analyses of niches and niche construction in macrobes, understanding microbial communities will also require large-scale analyses of their interactions with the physical and chemical environments they inhabit, meaning that metagenomics and dynamic ecosystem models need to be integrated with geomicrobiology (Doney et al. 2004; Croal et al. 2004; Newman and Banfield 2002; Oremland et al. 2005).

One way for philosophers to approach these implications is via a project sometimes called the 'second human genome project'. It begins with an inventory and analysis of *all* the DNA in a human body in order to gain a better understanding of the largely unexplored human biome (Relman and Falkow 2001). Because there are estimated to

be at least 10 times as many microbial cells in our bodies as there are human somatic and germ cells [13] (Savage 1977; Berg 1996), as well perhaps 100 times more genes (Xu and Gordon 2003), a full picture of the human organism sees it as a 'composite of many species and our genetic landscapes as an amalgam of genes embedded in our *Homo sapiens* genome[14] and in the genomes of our affiliated microbial partners (the microbiome)' (Bäckhed et al. 2005; Lederberg, in Hooper and Gordon 2001). Our microbiome functions as an additional 'multifunctional organ',[15] carrying out essential metabolic processes that we have never evolved for ourselves in the narrow single-organism or single-genome sense (Xu and Gordon 2003). These activities in this dynamically changing community are likely to involve sophisticated biochemical signalling and modulation of gene expression, not just within the microbial communities but between them and the host (Hooper et al. 1998; 2001).

A metagenomic analysis of the human biome would avoid some of the problems attached to traditional investigations that assume unique and separable contributions from individual species (Hooper et al. 1998). Not only would such a project involve a far greater understanding of the biodiversity existing in the ecological niches provided by human bodies, but it would also lead to a considerably more thorough understanding of how human health, disease resistance, development and evolution have depended and continue to depend on interactions with microbes.[16] Even philosophers of biology who remain sceptical about the project of metagenomics and the concept of the microbial metaorganism should note that there are obvious risks in any attempt to understand the nature of an organism abstracted from its inseparable microbial context.

There is, at any rate, a great deal of conceptual work to be done in metagenomics and philosophers need to ask some basic questions about the ontological status of metagenomes and metaorganisms, particularly whether the community organism is more fundamental than the individual organism. To answer this question we would have to clarify whether metagenomes are spatially defined by nature or whether they are they just entities defined by the spatial limits microbiologists have found temporarily convenient to impose. This issue of definition is very similar to the one that surrounds the notion of community.[17] Macrobial ecologists have tended to shy away from any notion of communities having functional properties analogous to organisms because clear spatial and temporal boundaries appear to exist only at the level of the individual organism (Parker 2004; Looijen 1998). Communities simply do not have firm boundaries or discreet forms due to the continuous nature of the environmental conditions that shape them. Consequently, communities are defined very loosely, usually as groups of populations in a place the ecologist happens to be studying (Underwood 1996; Collins 2003). The notion that communities might have emergent properties that individuals do not is considered by many ecologists to be misleading and irrelevant (Underwood 1996). This 'boundary problem' for communities of plants and animals is even worse for microbes, which are generally considered to be globally distributed and always potentially interacting (Finlay and Clarke 1999).

How serious is the boundary problem for metagenomes and metaorganisms? First of all, hard boundaries are not necessarily connected to ontological fundamentalness.

Philosophers of biology willing to accept the thesis of species as individuals in conjunction with even limited hybridity should have no difficulty acknowledging this point. There is, moreover, empirical work reversing expectations about organismal integrity and microbial ubiquity.[18] It may be sufficient to conceptualize microbial systems as poorly bound individuals that have some 'un-organism-like properties' (McShea 2004) while still possessing many organismal (or proto-organismal) characteristics. So far, metagenomics is not positing a level of physiological integrity to microorganismal communities that is wholly equivalent to individual organisms, but it does appear to be hypothesizing that communities have properties that are not reducible to simple interactions between individuals who just happen to have blundered together. The degree to which community structure persists over time and space may be unique to each community, but needs further research. If the community system is posited as more ontologically fundamental than the individual components, then its causal properties will have detectable and important influences on the constituents. The research mentioned above that is concerned with understanding the multicellularity of bacterial communities gives good reasons to follow this issue further and suggests avenues of research that metagenomic data could be directed towards (rather than simply scaling up and hoping to find emergent properties that way). There is a great deal of conceptual work to do in this area, and we anticipate philosophers of biology will be able to make useful contributions to it – especially in regard to the implications of metagenomics for evolutionary theory.

2. Evolution

Evolution has, for the most part, been about microbes, and all the most major evolutionary questions revolve around unicellular life: how life began, how prokaryotes evolved to eukaryotes, and how the transition from unicellular to multicellular life was accomplished. The philosophy of biology is, of course, interested in these issues but primarily as a backdrop to its evolutionary focus on multicellular organisms. The neglect of microbes can be particularly striking in what is in many respects the most exciting topic in philosophy of evolution, evolutionary developmental biology or 'evo-devo'. For example, Robert (2004: 34) writes: 'Development is what distinguishes biological systems from other sorts of systems, and it is the material source of evolutionary change'. Since microbes, though they go through cycles of internal reorganization do not, in the normal sense, develop at all, it would appear that on this view they are not biological systems and apparently could not have evolved. Of course, as we have been speculating, it might turn out that microbes are not biological systems, but only parts of biological systems (metaorganisms) and it may be that only as such could they have evolved. But it is doubtful whether metaorganisms have the kind of developmental properties that this vision requires, and certain that this is not the idea that Robert intended. Surely it reflects an oversight, but one we think is very telling of the tendency for philosophy of biology to focus myopically on macrobes. Evolutionary microbiology in general and metagenomics in particular offer a variety of findings that enrich and challenge standard evolutionary theory.

A long-standing debate in the philosophy of biology has been about the units and levels of biological organization on which selection acts. A key divide has been whether selection operates in a privileged way on genes and organisms, or whether it also operates (not exclusively) at group and other levels (Brandon and Burian 1984; Sober and Wilson 1994). Although considerable conceptual progress has been made over the last two decades (Lloyd 2000; Brandon 1999; Okasha 2003), prokaryote communities have hardly ever been used as illustrations or objects of analysis in the debate.[19] One of the obvious questions the discussion of community function or metaorganisms raises is whether these apparently coevolved relationships and community-level properties are selected for, or whether such selection can be fully accounted for at the individual gene-organism level (Collins 2003; Whitham et al. 2003). Can such entities as metagenomes or metaorganisms be conceived of as units of selection? Is there selection between metagenomes that sustain communities of varying fitness or is the selected unit genomic or sub-genomic? Is there competing selection of individual cells and genes that threatens the cooperation achieved at the community level? Systems involving commensal microbes and macrobes amplify these questions but probably do not complicate them. These complex communities are probably, in fact, *the* standard unit of selection.

From a multilevel selectionist perspective, if selection operates at the community level, the community is analogous to an organism and individual organisms are analogous to genes (Wilson and Swenson 2003) or, perhaps better, cells. The interaction between these levels and the environment produces phenotypic properties at the community

level that allow the whole community to survive selection and reproduce itself advantageously in relation to other communities without the relevant properties. For selection at this level to have cumulative effects, communities would have to be cohesive wholes that interacted directly with their environments in a way that led to observable differences in replication success (Sober and Wilson 1994; Wilson 1997). So far, we have not noticed metagenomics voicing such a community-level account of selection and no research is being directed specifically towards it. The implications for multilevel selection are there, however, and metagenomics may provide new material for a better understanding of how selection operates as well as for identifying the mechanisms that allowed a hierarchy of biological organization to evolve in the first place (Okasha 2004; 2003).

Many of the questions we would want to ask about group-level selection happening now would be questions even more pertinent to the emergence of multicellular organisms. For the multicellular organism to have become an individual in its own right (as opposed to an aggregation of cells), selfish tendencies of single cells would have had to have been regulated and cooperative interactions promoted (Michod 1997a; 1997b; Buss 1987; Okasha, 2004). Maynard Smith and Szathmáry's (1995) account of major evolutionary transitions specifies that entities that replicated independently before the transition can replicate only as part of the larger whole (or next level of organization) afterwards. Okasha (2003) and Michod (1997a, b) make this point more subtly and argue that the transition to multicellularity would begin on the basis of group fitness

equalling average (lower-level) individual fitness, but that higher-level fitness would eventually decouple from component fitness as the transition proceeded.

It may be that while this point is basically correct, its formulation suffers from a shortsightedly macrobial perspective. The components of an integrated community would not be capable of independent replication, not because replication had become a specialized function but because the various components could only function cooperatively. Sequestered reproduction grounds one very interesting form of cellular cooperation, but perhaps we should avoid thinking of it as the only possible form. In other words, we might speculate that macrobial multicellularity (like organelles in eukaryote microbes) is just a frozen, less flexible, form of bacterial multicellularity. Prokaryote cell differentiations can dedifferentiate whereas metazoan multicellularity is irreversible (Jefferson 2004). The multicellularity we commonly think about had to be selected for, to be sure, but in the long run of evolution it is likely to be much less well able to adapt to major changes in environmental conditions, such as atmosphere. Or, if it does adapt, this may be very much dependent on the more diverse capacities of microbial commensals.

One general point we want to stress is that we should avoid seeing the multicellular transition as representing unambiguous progress, as a move from the primitive to the sophisticated. In many ways, microbial communities seem to work a lot better than macrobes do. No doubt the key to understanding how macrobes evolved at all is to locate more clearly what it is that they do better than microbial communities[20] (unless,

indeed, we should see macrobes in a neo-Dawkinsian way, as primarily vehicles for the billions of microbes that live in the many niches they provide).

Conversely, we need to resist the temptation to see microbes as primitive precursors of macrobes. Indeed, we need to face the fact that much of our evolutionary theory is grounded in features peculiar to macrobes and has questionable relevance to microbial evolution — which is to say, by far the largest part of all evolution. It may also be, in a real sense, the most important part of evolutionary history. For it is clear that the basic machinery of life evolved in microbes prior to what might, in relative terms, be seen as no more than a severe narrowing and slight diversification of the applications of that chemistry in macrobes. And, of course, it is only due to ancient prokaryotic mergers that there are eukaryotes at all (Margulis 1970),[21] and large-scale gene exchange between all three domains – even from eukaryotes to prokaryotes (de la Cruz and Davies 2000) – makes the prioritization of any domain a senseless exercise.

More fundamental even than these questions about major evolutionary transitions is the need to reflect on the mechanisms by which microbial communities adapt and evolve. Microbial populations exhibit much more rapid rates of evolutionary change than do their macrobial equivalents, the variety of dynamics and mechanisms of evolution is more diverse, and extinction means something quite different if indeed it has any relevance at all to microbes (Stahl and Tiedje 2002; Lawrence 2002; Weinbauer and Rassoulzadegan 2004; Staley 1997). Most importantly, the genetically isolated lineage, often conceived of as the fundamental unit of evolutionary theory, may have no real

analogue in the microbial world. The philosophy of evolutionary biology must take account of the rapidly growing body of work in microbial phylogeny on horizontal or lateral gene transfer. Metagenomics has already extended the earlier findings of comparative evolutionary genomics and further exacerbated the problems it uncovered in the dominant eukaryo-centric paradigm of vertical inheritance and mutation-driven species divisions that give rise to a single tree of life (Allen and Banfield 2005; Rodríguez-Valera 2002; 2004; DeLong 2002b; Stahl and Tiedje 2002; Gogarten and Townsend 2005; Doolittle 2002; 1999). A field that is sometimes called 'horizontal genomics' investigates the plethora of mobile genetic elements (the 'mobilome') available to microbial communities and how they parcel out the metagenomic resource co-inherited by interacting populations (Frost et al. 2005; Smets and Barkay 2005).

Gene cassette metagenomes provide an interesting example of how such study is being pursued. Gene cassettes are the smallest known units of mobile DNA. They, and the integrons in the host genome that allow the cassettes to be inserted or excised, are efficient mechanisms for the movement and expression of genes within and between species, and are implicated heavily in antibiotic resistance (Michael et al. 2004; Holmes et al. 2003). Gene cassettes were originally studied individually but a metagenomic perspective allows them to be studied as a 'floating' evolutionary resource of high diversity and widespread activity that exists independently of individuals and is likely to have a high impact on bacterial genome evolution (Holmes et al. 2003; Michael et al. 2004). Although the extent, types and precise effects of this mobile resource still require much more research, the conceptual implications for evolution are already obvious

(especially because they back up extensive work done on lateral gene transfer and recombination processes). Rather than focusing on individual organismal lineages, such metagenomic studies enable a shift in scientific and philosophical attention to an overall evolutionary process in which metagenomes contribute to the differentiation of ecosystem interactions. Experimental and modelling work on gene exchange among in situ communities in biofilms will supplement the retrospective gene exchange analyses of evolutionary genomics with an 'as it happens' perspective that will enrich metagenome understanding (Sørensen et al. 2005; Springael and Top 2004), although a great deal more work needs to be done to understand the role of gene transfer in ecosystems (Thomas and Nielsen 2005).

It might be possible in principle to construct evolutionary models in which microbial clones play a similar role to the familiar macrobial lineages. But even apart from the great diversity of clonal structure exhibited by different microbial taxa, there are some serious difficulties with such models. The most obvious is time scale. Microbial clones have lifespans of hours or days rather than the thousands of years typical of macrobial lineages. This suggests a need for higher level models if any sense is to be made of long term evolutionary change. It further needs to be decided how the beginning and end of a clone are to be defined for this purpose, especially in light of a large body of evidence that shows little true or enduring clonality in most bacterial populations (Maynard Smith et al. 1993; Maynard Smith et al. 2000). At any rate, the failure of microbial clones to exhibit the genetic isolation that provides the ultimate motivation for treating macrobial lineages as units of evolution appears to undermine any motivation

for this approach. The prevalence of mobile genetic elements moving between microbial units again points to a focus on larger units within which these movements take place.

This point suggests a slightly different formulation of the question raised earlier about the boundaries of metagenomic units. If it turns out that the lateral circulation of genetic material takes place within reasonably clearly delineated microbial communities, it may be useful to consider these as metaorganisms and, in turn, as potential units of selection. Surely such relative isolation will apply to communities defined by their residence in, for example, a particular human gut. Whether the same applies to soil bacteria, say, is another matter. If not, either microbial evolution is limited to more peripheral, isolated environments or, more likely, we will need to go further from traditional macrobial models in search of an adequate understanding of microbial evolution.

All of these pattern and process issues highlighted by metagenomics are topics that the philosophy of biology has to take notice of unless it restricts itself to a narrow kingdom-specific understanding of evolution. Microbial genomics and metagenomics have evolutionary implications that reach into the most basic representations of evolution since they make clear that most of life and its history cannot be simply configured as a tree-like pattern of evolutionary outcomes (Doolittle 2005). This realization makes yet further deep inroads into the philosophy of biology because of its extensive implications

for microbial taxonomy, the units of taxonomy, and the philosophical appreciation of biodiversity.

3. Taxonomy and biodiversity

Taxonomy

Identifying categories of organisms is central to the task of understanding the diversity of past and present forms of life and the evolutionary relationships between them. While the philosophy of biology has often recognized prokaryote classification as a special case (e.g.: Sterelny 1999; Hull 1987a; Wilkins 2003), it has paid the issues involved hardly any attention and continues to believe that evolutionarily defined categories of organisms can be represented as bifurcating lineages that compose a tree of life. A variety of concepts have been proposed to define the species that make up this tree, but all of them prove unsatisfactory when gene exchange and genomic heterogeneity are brought into the picture. Prokaryote taxa simply refuse to show the clear, consistently definable characteristics commonly associated with eukaryotic species and classification schemes (Roselló-Mora and Amann 2001).

The key issue from a microbial genomics perspective is whether to think of prokaryote taxa as continua or as discrete clusters of species-specific genetic diversity (Konstantinidis and Tiedje 2004; Doolittle 2002; Lan and Reeves 2001). Although the biological species concept (BSC) has never found much purchase in microbial

systematics[22] because of its exclusion of asexual reproduction and difficulties in coping with gene transfer between evolutionarily distant lineages (Maynard Smith 1995; Cohan 2002; Dupré 1999), there is still a quest by many microbiologists for an appropriate evolutionary or phylogenetic definition (Roselló-Mora and Amann 2001). In its simplest form, this simply means species are defined by common ancestry and must be monophyletic. Usually, however, this basic concept is accompanied by assumptions about which molecules are more reliable bases of such phylogenetic inference, and ribosomal DNA sequence is generally considered to be the prime candidate for divulging 'natural relatedness groups, the phylogenetic divisions' (Hugenholz et al. 1998; Ward 2002).

As we outlined in the background section, the role of 16S rRNA gene sequence as the ideal phylogenetic marker has been undermined by conflicting genomic evidence, which has also damaged the idea of a single true marker for organismal evolutionary history. Other microbiologists emphasize the importance of ecological forces on populations, with 'ecotypes' (equivalent to strains) being the product of ecological (but not reproductive) divergence (Cohan 2002; Palys et al. 1997; Gevers et al. 2005). Pragmatists, generally more convinced of the extent and implications of gene exchange, use the word 'species' as a purely practical term that means 'assemblages of related organisms for which microbiologists have attached specific names rather than natural kinds' (Gogarten et al. 2002). These are 'species-like' entities (Rodríguez-Valera 2002) whose classifications are created by classifiers, not nature.

Popular operational measures reflect the mixture of concepts and conceptual problems at work in microbial systematics. The currently predominant operational measure of where the boundary falls between prokaryote species is below a 70% rate of DNA-DNA reassociation in hybridization tests of the total genomic DNA of two organisms (Roselló-Mora and Amann 2001; Dijkshoorn et al. 2000). This crude measure of genomic distance is commonly considered equivalent to 97% rRNA identity. The first value was chosen because it appeared to map onto phenotypic clusters for no known evolutionary reasons; the second because it conveniently mapped onto the 70% measure (Cohan 2002; Lan and Reeves 2001). As well as the fact both measures ignore apparently important genomic differences, there is no evolutionary reason why 70% DNA-DNA similarity values should be a species boundary, nor for 16S genes to be exempt from transfer or recombination (Boucher et al. 2001; Lan and Reeves 2001; Palys et al. 1997).[23] Moreover, the correlation between DNA-DNA reassociation and 16S sequence varies in different genera, and it is well known that the 16S gene lumps together functionally diverse strains (Staley and Gosink 1999; Kämpfer and Rosselló-Mora 2004).

An influential proposal designed to overcome these inconsistencies is the quasi-official (American Society of Microbiology) species definition. It combines genomic, phylogenetic and phenotypic approaches into a pragmatic and polyphasic taxonomic framework: ‘a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics, and is diagnosable by a discriminating phenotypic property’ (Roselló-

Mora and Amann 2001: 59). In practice, however, any such practical species measure is still anchored phylogenetically by the 16S rRNA gene (Young 2001; Dijkshoorn et al. 2000) which is seen as a proxy for natural units and their boundaries and helps overcome the discomfort of many microbial systematists in regard to 'non-natural' classification concepts and methods (e.g.: Coenye et al. 2005).

While some microbiologists believe that genomic science will ultimately enable an answer to the question of whether a natural entity of species exists (e.g.: Stahl and Tiedje 2002; Kämpfer and Rosselló-Mora 2004; Konstantinidis and Tiedje 2005), it appears that genomic data can be made to fit as many theoretical frameworks as are developed. Some microbial taxonomists see the species problem as one of relying too heavily on purely genomic data. They believe it will be resolved by a better pluralistic combination of data and methods (Young 2001; Vandamme et al. 1996). Others see the problem as one of limited genomic scope in existing phylogenies. It can be remedied by methods that employ greater amounts of genomic data, either as genome trees (Wolf et al. 2002) or supertrees (Daubin et al. 2001). The post-16S solution often involves some notion of a 'core' species genome (e.g.: Ward 1998; Lan and Reeves 2001) that can serve as an equivalent of a closed gene pool and thereby support the BSC. A core genome, however, remains an elusive and multiply-defined phenomenon (Boucher et al. 2001).

If, as we have suggested above, the individual microbe is not the fundamental ontological unit in microbiology, then it should be no surprise that attempts to find a

division of individual microbes into natural kinds is doomed to failure. Metagenomicists should be well prepared for the discovery that species genomes or 16S phylotypes fail to capture the way microbial life has organized itself, or indeed, that microbial life and evolution does not lend itself to a monistic, consistently applicable species concept that allows evolutionary history to be represented as one true tree of life.

Many further questions remain in this area. What else does metagenomics imply for microbial taxonomy? Or what can taxonomy do for metagenomics? Is there potential for a taxonomy of metagenomes or metaorganismal lineages, or do these entities have little biological and no taxonomic significance because of their weak boundaries and evolutionary lability? Should genomic identity or functional role guide the classification of participants in community systems? Finally, if we let the idea of the metagenome as a dynamic community resource further undermine the notion of stable species boundaries, what are the implications for how we understand biodiversity?

Biodiversity

Although it is common knowledge that microorganisms have a far longer evolutionary history and a biomass estimated to be greater than that of multicellular organisms[24] (Whitman et al. 1998; DeLong and Pace 2001), there is no general appreciation of microbial biodiversity as there is of macrobial biodiversity. Microbial diversity is generally given short shrift by biodiversity studies and philosophers of biodiversity (Ehrlich and Wilson 1991; Loreau et al. 2001; Sarkar 2002; Oksanen and Pietarinen 2004; Nee, 2004; May 1994), mostly because of methodological and technical

limitations. Microbiologists have long known that their understanding of microbial diversity has been restricted by technology and a health- or agriculture-based bias towards pathogens. Microbes' enormous diversity of habitats, metabolic versatility and physiological adaptability are only beginning to be mapped by genomic methods. Genomics-driven estimates have risen to as many as 10^7-10^{12} microbial 'species', of which fewer than 36,000 are indicated by rRNA sequence analysis (Schloss and Handelsman 2004) and only 7,800 of those are named and described[25] (Kämpfer and Rosselló-Moro 2004; Dykhuizen 1998).

Simple numerical comparisons of eukaryotic and prokaryotic diversity by species counts or estimates are inadequate for several reasons. As we have just seen, there are deep conceptual problems in defining the microbial species. If eukaryote species were designated by the same broad genomic hybridization criteria that prokaryote species are, then groups such as humans, chimpanzees, orangutans, gibbons, baboons and lemurs and would all belong to the same species (Staley 1997). Environmental genomics is centrally concerned with escaping these limitations, although it still relies heavily on ribosomal gene sequence to do so. One of the early benefits of metagenomics is expected to be its contribution to a broader and deeper understanding of microbial diversity.

At present, metagenomics is largely occupied by cataloguing that diversity, but as its research scope stretches to include multilevel interactions and processes, the object of study becomes biodiversity in an extended functional sense. The metagenome contains

a ‘staggering’ amount of new information about microbial genomes, population structure and the processes operating within and between genetic and phenotypic groups (Reisenfeld et al. 2004; Venter et al. 2004; Holmes et al. 2003; Streit and Schmitz 2004; DeLong,2005). It thus provides a means by which to begin analysing microbial diversity from a functional point of view.

Appropriate ecological assessments of biodiversity need to be able to take into account the variability of microbial populations as well as the relationship between community structure, biogeochemistry and ecosystem function (O’Donnell et al. 1994; Stahl and Tiedje, 2002; Ward 2002; Buckley 2004a). Deeper analyses of biodiversity also need to generate explanations of ‘the tempo, mode and mechanisms of genome evolution and diversification’ in relation to higher-order biological and ecological processes (DeLong 2004b; Falkowski and de Vargas 2004). Metagenomics enables further steps to be taken towards reconceiving the object of biodiversity studies as processes rather than things. As understanding of the role of microbial communities in ecosystem function grows, and microecological studies are integrated with macroecological, it is likely that philosophical and practical arguments for microbial conservation – not recognized at all in the philosophy of conservation – will also develop (Colwell 1997; Staley 1997). Clearly, these are not straightforward research programmes that will give simple answers about biodiversity, but they are aspirations towards understanding complex phenomena for which technology and tools of analysis are beginning to develop. Philosophical analysis, even at this early stage, could make important contributions to framing the questions that are asked. Although we can only raise the questions and not

answer them, we believe that here again metagenomics opens up a new realm of questions for philosophy of biology.

Towards a more inclusive philosophy of biology

Even prior to recent developments stemming from the growth of genomic technology, philosophy of biology has been culpable in its failure to take serious account of the microbiological realm. Today this omission is inexcusable. Metagenomics, in particular, has brought together a range of diverse and interconnected microperspectives that have fundamental importance for how we understand life. These reconceptualizations are not just a background development but a major transformation in understanding that needs to be reflected in the philosophy of biology. Although from an epistemological point of view it could be argued that metagenomics is not so much revolutionary as a continuation of earlier dramatic reorientations in microbiology, the field leads to radical reappraisals of the nature of boundaries between biological entities and the organization of life itself. Indeed, these ontological transformations would appear to embody the fullest realization of a systems approach to biological phenomena, even though it remains to be seen whether true systems understanding can be actualized in any form of biological practice (O'Malley and Dupré 2005).

Philosophy of biology's lack of attention to metagenomics could be due to nothing more remarkable than the field's newness. It is quite probable, however, that metagenomics

will remain largely invisible to philosophy if the latter's systematic lack of interest in microbes continues. It is rare, even in classification and species discussions, for philosophers to invoke microbial phenomena. Philosophical discussions of biodiversity produce only apologies for ignoring microbial biodiversity (e.g.: Lee 2004). Even in philosophical debates about evolutionary processes, little notice is taken of microbes except when they are placed as backdrops to what is in truth merely 'the sideshow of metazoan evolution' (Sterelny and Griffiths 1999: 307).

Throughout this paper, we have given numerous reasons why microbes and microbiology cannot be ignored, no matter what forms of life are being scrutinized and even if humans are given some kind of dominant species status. The natural history of life on earth was and always will be 'the age of bacteria' (Gould 1994),[26] and certainly life as we know it depends primarily on microbial functions. Just in terms of biological science, many of the most significant (Nobel prize-winning) achievements of the last few decades have been based on prokaryotes and their phages. These include fundamental knowledge about biosynthetic pathways, gene activity and regulation, the genetic code, DNA replication, transcription and translation, mutagenesis and repair – not to mention the tools of restriction enzymes, reverse transcriptase and DNA polymerase (Moxon and Higgins 1997; ASM 2005). At the very least, a philosophy of biology that excludes microbiology can be only a partial and skewed philosophy of life and how it is studied.

Finally, it might be worthwhile hazarding a guess as to why the philosophy of biology has been so willing to ignore microbes and microbiology. Candidate reasons could be

the intractability of microbial analysis, ignorance, authority, invisibility, and a progressive view of evolutionary history. Intractability of analysis (microbiology's difficulties in coming up with a natural classification system and measures of diversity) is not a good reason because the difficulties of microbial classification and species conceptualization might just as easily have stimulated philosophical scrutiny and contributions. It is not a simple matter of ignorance either, because many philosophers of biology are at least aware enough to sweep microbes aside. Does philosophy of biology focus on metazoans simply because of some old attributions of status to zoology and animals (over botany and plants as well) that haven't been challenged? An even more basic explanation could be a cognitive bias towards larger, more visible phenomena – the same reason Sean Nee (2004) gives for the public indifference to microbes. It would seem odd, however, for philosophers to be involved in debates about the obscure minutiae of other biological findings but then to ignore a whole field of phenomena whose importance has been obvious since the microscope.

Some scientists perceive 'an unspoken philosophy of "genomic supremacy"' (Relman and Falkow 2001: 206) that is accorded to more complex animals because of genome size and number of predicted genes. If this were strictly true, then cereals, amphibians and some amoeba would be ranked higher and receive more philosophical attention than mammals, which is patently not the case. Any unspoken philosophical ranking of life forms and their study would have to be more generally about human supremacy (Paabo 2001) and comparative genomics is more likely to challenge such a notion than to support it.

Taking this explanation of human supremacy further, Stephen Jay Gould (1994) sees general indifference to microbes as part of the 'conventional desire to view history as progressive, and to see humans as predictably dominant' thus leading to overattention to 'complexifying creatures'. This view places at the centre of life a 'relatively minor phenomenon' instead of the most salient and enduring mode of life known to this planet. Is it possible that philosophers, usually amongst the first to condemn notions of progressive evolution, are under the influence of this view of the history of life when they ignore microbes? Perhaps a more charitable interpretation is that the discontinuity of life forms implied by the prokaryote-eukaryote division (Stanier and Van Niel 1961; Olsen et al. 1994; Sapp 2005; Woese 2005) and the emphasis of negative characteristics of prokaryotes (no nucleus, no internal membranes, small size) gave rise decades ago to a generally unchallenged notion amongst philosophers that microbes were less interesting than their (assumed-to-be) categorically different multicellular descendants. That this notion is maintained despite the growth of knowledge and theory in microbiology means that adherence to a bad habit is the only reasonable explanation for the reluctance of philosophers of biology to deal with microbes. In that case, metagenomics might provide just enough of a conceptual kick to initiate a wider range of thinking in the philosophy of biology and perhaps even initiate a philosophy of microbiology.

NOTES

[1] The word macrobe has, unsurprisingly, been used before (e.g.: Postgate 1976; Dixon 1994), but the usage has not been widely adopted. We distance our use of it from any resonance with C.S. Lewis's in his book *The Hideous Strength* (1945), where macrobe refers to a class of malign spirits.

[2] See Simpson and Rogers (2004) for a much less traditional division of eukaryote kingdoms.

[3] Although there are numerous disputes about admissible data and interpretations, common dates for prokaryote origins are 3.8-3.5 billion years ago, followed by the first eukaryote microorganisms 1.5-2.0 billion years later, with the first multicellular eukaryotes emerging around a billion years after that (see Waggoner 2001; Kerr 2005; Carroll 2001; Nisbet and Sleep 2001; Martin and Russell 2003).

[4] See the 'Biodiversity' section for details.

[5] There are, of course, exceptions to this tendency. Amongst them are Jan Sapp (1987; 2003), whose historical work on microbiology delves deeply into the philosophical issues of the discipline; Carol Cleland (Cleland and Copley, forthcoming), who has written about alternative definitions of life with particular reference to prokaryotes; and Kim Sterelny (2004), who proposes the transmission of bacterial symbionts as an inheritance system. We are sure there must be others, but detailed philosophical attention to microbes is rare.

[6] There are some exceptions to the 'visiblity' rule.

[7] We continue using the convenient label of prokaryote throughout this paper because it does usefully describe both archaea and bacteria in terms of cellular and genomic size and organization. See Walsh and Doolittle (2005) for a better argument along these lines.

[8] 'It must be remembered,' sniffs Mayr (1998: 9721), 'that Woese was not trained as a biologist and quite naturally does not have an extensive familiarity with the principles of classification.'

[9] The main application and commercial focus of metagenomics is the discovery of 'novel natural products' such as enzymes and biocatalysts (Voget et al. 2003; Lorenz and Eck 2005; Lorenz and Schleper 2002; Schloss and Handelsman 2003; Daniel 2004).

[10] These abilities would appear to fit general definitions of multicellularity: 'By a multicellular organism we understand one in which the activities of the individual cell are coordinated and the cells themselves are either in contact or close enough to interact strongly' (Kaiser 2001: 104). Users of this definition tend to reject bacterial colonies as candidates for multicellularity on the grounds they lack overall coordination of function (Kaiser 2001; Wolpert and Szathmáry 2002). The research above, however, shows the rejection to be too sweeping particularly if Michod's definition of organisms as 'groups of cooperating cells related by common descent' (1997a: 608) can be stretched to accommodate metagenomes as potentially common heredity.

[11] These communities can include eukaryote hosts and the bi-directional modulation of gene expression in host and commensals (Shiner et al. 2005).

[12] See Redfield (2002) for a discussion of the high evolutionary costs of interspecies quorum sensing and an argument against the 'cooperation' interpretation. However, the evolutionary bargain seeking between cells within traditionally-conceived organisms and control of selfishness is as much of a problem (Michod 1997a) – probably no more easily solved than in a cooperating bacterial community (see below, in text).

[13] Just the E. coli population in a single human is comparable to the entire human population Staley 1997).

[14] The human genome (in the traditional, narrow, sense) appears to contain some microbial DNA (a disputed amount) that was transferred directly into vertebrates rather than being inherited from non-vertebrates (Salzberg et al. 2001), as well as retroviral DNA – about 1% of the genome (Löwer et al. 1996).

[15] The metabolic activity of just the gastrointestinal bacteria in a human is believed to be equal to that of the liver – the most metabolically busy organ in the human body (Berg 1996).

[16] Every eukaryote is, in fact, a superorganism, composed of chromosomal and organellar genes and a multitude of prokaryote and viral symbionts – a 'symbiome' (Lederberg, 2000, in Sapp 2003). See McFall-Ngai (2002) for a discussion of the influence of bacteria on animal development programmes. Other complex and highly organized commensal and mutualist associations would also be fascinating candidates for metaorganismal analysis (e.g.: termites and their intestinal microflora [Blume and Friederich 2000]), but presenting such studies in terms of the human biome might make microbial systems especially salient for philosophers.

[17] Thanks to Christina Matta (Handelsman lab, University of Wisconsin) for this point.

[18] The omnipresence of genetic exchange in microbial communities obviously shows organism boundaries to be much more permeable than might have been thought. And, although it has long been presumed that 'everything is everywhere' in relation to microbial distribution, meaning that microbes have *no* biogeography (Finlay and Clarke 1999), recent studies taking a more extensive and finely resolved genomic perspective have found that communities of free-living bacteria and archaea in hot springs and soils do actually have geographic limits at the strain level (Papke and Ward 2004; Whitaker et al. 2003; Cho and Tiedje 2000).

[19] See, for example, the table in Goodnight and Stevens (1997). Parasite populations are popular illustrative examples, but they are usually metazoan parasites (e.g.: Sober and Wilson 1998). The myxoma virus infection of rabbits used in the earlier stages of the debate (e.g.: Lloyd 1989) is an exception to the focus on multicellular organisms.

[20] Bonner (1998) points out that it is likely early multicellular clusters may have had no adaptive advantages.

[21] See Martin and Russell (2003) for an evaluation of competing hypotheses on eukaryote origins.

[22] See below for genomic attempts to apply this concept to microbial systematics.

[23] And indeed, they are not (see Boucher et al. 2001, for citation of evidence).

[24] A standard estimate is 4-6 x 10^{30} prokaryote cells (Whitman et al. 1998). The greater biomass estimate excludes the extracellular material composing plant biomass.

[25] Versus over a million named plants and animals (Staley and Gosink 1999).

[26] Some important evolutionary biologists are entirely unconvinced by such arguments. Bacteria can claim only biochemical expertise and they occupy only leftover environments. Macrobes, particularly metazoans, are much more 'obviously' biologically interesting (e.g.: Conway Morris 1998). Our paper is trying to challenge all the assumptions in such arguments.

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