Biology and Philosophy (2007) 22:155–191
DOI 10.1007/s10539-006-9031-0

Size doesn't matter: towards a more inclusive philosophy of biology

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Received 12 October 2005; accepted in revised form 22 March 2006

Key words: Biodiversity, Evolution, Macrobes, Microbes, Microbiology, Multicellularity, Ontology, Prokaryotes, Taxonomy

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. We set out a number of recent developments in microbiology – including biofilm formation, chemotaxis, quorum sensing and gene transfer – that highlight microbial capacities for cooperation and communication and break down conventional thinking that microbes are solely or primarily single-celled organisms. These insights also bring new perspectives to the levels of selection debate, as well as to discussions of the evolution and nature of multicellularity, and to neo-Darwinian understandings of evolutionary mechanisms. We show how these revisions lead to further complications for microbial classification and the philosophies of systematics and biodiversity. Incorporating microbial insights into the philosophy of biology will challenge many of its assumptions, but also give greater scope and depth to its investigations.

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

[1]The word macrobe has 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.

of choice of defining features, neither of these categories would normally be attributed much biological coherence.

In general, any organism too small to be seen without a microscope is called a microbe or microorganism, even though many of them are visible when clustered together (e.g., mould and algae filaments).[2] Microbes comprise two of the three superkingdoms,[3] Bacteria and Archaea, as well as single-celled eukaryotes (protists and yeasts) and viruses. Viruses, because they have no cells or metabolic function and require other organisms to replicate, tend to be placed in a grey zone between living and non-living things (or organisms and chemicals), but their evolutionary history, involvement with prokaryotes and eukaryotes, and some surprising biological capacities (Luria et al. 1978; Raoult et al. 2004; Villarreal 2004a) make it difficult to dismiss them as non-living. We will focus on bacteria and archaea in this paper, though many fascinating stories and philosophical complications could also be drawn from viruses and protists (e.g., Sapp 1987; Corliss 1999; Nanney 1999; Villarreal 2004b). Bacteria and archaea – until the 1970s considered under the single classification of bacteria – are now 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; Allers and Meverech 2005).

Macrobes comprise the remainder of the Eukarya, the kingdoms Animalia (including the Metazoa), the Fungi and the Plantae.[4] The distinction between macrobes and microbes is not entirely sharp: various social single-celled organisms, both prokaryotic and eukaryotic, such as the myxobacteria and cellular slime moulds, have long-recognized claims to multicellularity. We frame our argument round this distinction for two reasons, however. First, the macrobes are no more diverse a group than 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.

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

[2]There are some bacteria visible as single cells, most notably *Thiomargarita nambiensis*, which is a recently discovered spherical sulphur bacterium with a diameter of 750 μm (Schulz and Jørgensen 2001).

[3]Superkingdoms or domains are the highest levels of taxa. The third superkingdom is Eukarya or Eukaryota, of which protists make up a substantial proportion (see the following Note).

[4]A recent and less traditional division proposal for eukaryote kingdom divisions by Adl et al. (2005; Simpson and Rogers 2004) sets out six eukaryote kingdoms of which four are solely protists. Plants are part of Archaeplastida (which also contains single-celled algae) and animals merely a subset of Opisthokonta (which includes true fungi and several protist groups).

only about 545 million years ago (Carroll 2001; Conway Morris 2003).[5] Microbes have far greater metabolic diversity than macrobes and can utilize a vast range of organic and inorganic energy sources via numerous metabolic pathways (Amend and Shock 2001). They are deeply implicated in the geochemical development of the planet, from the formation of ore deposits to the creation and maintenance of the oxic atmosphere on which macrobes depend[6] (Kasting and Siefert 2002; Newman and Banfield 2002). They can thrive in conditions that are intolerable for most plants and animals.[7] Prokaryotes flourish in temperatures over 100 °C and at least as low as −20 °C. They colonize extremely acidic, alkaline, salty, metal-rich, radioactive, low-nutrient and high-pressure environments. They can be found in high-altitude clouds and on human artefacts in space, several kilometres deep in the earth's crust, as well as on and in every eukaryote organism alive or dead (Horikoshi and Grant 1998; Price 2000; Newman and Banfield 2002; Nee 2004). Just one gram of ordinary uncontaminated soil contains 10^{10} prokaryote cells which consist of as many as 8.3×10^{6} species (Gans et al. 2005). Microbial species diversity in all of earth's environments is only estimated but it exceeds all other life forms, as do estimates of their global cell numbers.[8] The natural history of life on earth was and always will be 'the age of bacteria' (Gould 1994).[9]

Even an exclusive interest in mammalian or human biology cannot justify ignoring microbes. There are estimated to be at least 10 times as many microbial cells in our bodies as there are human somatic and germ cells[10] (Savage 1977; Berg 1996), as well as 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

[5]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 Nisbet and Sleep 2001; Carroll 2001; Waggoner 2001; Martin and Russell 2003; Kerr 2005).

[6]See Bryant (1991) and Lloyd (2004; also Biagini and Bernard, 2000) for a discussion of whether there are any true obligate anaerobic eukaryotes.

[7]See http://www.nhm.ac.uk/research-curation/projects/euk-extreme/ for an overview of eukaryote extremophiles (organisms that favour extreme environments), which are far fewer and more restricted than prokaryote extremophiles.

[8]A commonly accepted estimate is $4–6\times10^{30}$ prokaryote cells in all habitats (Whitman et al. 1998) and $\sim4\times10^{30}$ viruses just in ocean waters (Suttle 2005). Even though microbial cells are usually much smaller than eukaryote cells, prokaryotes and viruses account for well over half the biomass on the planet (if the extracellular material of plants is excluded) and an even greater percentage (perhaps 90%) if only the oceans are considered.

[9]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.

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

our *Homo sapiens* genome[11] 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',[12] carrying out essential metabolic processes that we, in the narrow single-organism or single-genome sense, have never evolved for ourselves (Xu and Gordon 2003). Every eukaryote can, in fact, be seen as a superorganism,[13] composed of chromosomal and organellar genes and a multitude of prokaryote and viral symbionts (Lederberg, 2000,[14] in Sapp 2003). This multispecific interactionist perspective, apart from fostering a far richer understanding of the biodiversity existing in the ecological niches provided by human bodies, should also lead to a better understanding of how human health, disease resistance, development[15] and evolution have depended and continue to depend on interactions with microbes.

Despite the biological significance of microbes and the centrality of their study to some of the most exciting biology of recent decades (see below), the philosophy of biology has focused almost exclusively on multicellular life.[16] 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. 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, p. 307).

In our conclusion we speculate briefly on why this has happened. Our main aim in this paper, however, is to argue for an end to this myopia. We aim to show the radical revisions new understandings of microbes force

[11]The human genome (in the traditional, narrow, sense) appears to contain some microbial DNA (an initially exaggerated but still not clearly established amount) that was transferred directly into vertebrates rather than being inherited from non-vertebrates (Genereux and Logsdon 2003; Iyer et al. 2004), as well as an abundance of retroviral DNA (Griffiths 2001; Bromham 2002). A call for a research programme named 'the second human genome project' argues for an inventory and analysis of *all* the DNA in a human body in order to gain a better understanding of the system of interactions between humans and microbes (Relman and Falkow 2001).

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

[13]Prokaryotes are similarly occupied by phages (bacterial viruses), which conduct a range of processes with the cellular machinery of their hosts.

[14]Lederberg's neologism for this community organism is 'symbiome'.

[15]See McFall-Ngai (2002) for a discussion of the influence of bacteria on animal development.

[16]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, 2005), 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 our general point – that detailed philosophical attention to microbes is rare – still stands.

upon some long-established ways of thinking in the philosophy of biology, specifically with respect to ontology, evolution, and taxonomy (including biodiversity). We will start with outlines of some recent developments in microbiological understandings of sensory capacities, communication processes and gene transfer, and show how these present fundamental challenges to traditional ways of thinking about microbes as primitive individual cells.

Microbiology: a brief history

Early microbiology and the pure culture approach

The history of microbiology begins with the invention and development of the microscope in the late sixteenth and early seventeenth century, but it took a considerable time for any deep understanding of microbes to develop. Their long-hypothesized association with illness, fermentation and food spoilage became an important topic of investigation in the late 1700s. In the early 1800s the stage was set for the first 'golden age' of microbiology with experimental tests of the spontaneous generation hypothesis, followed some decades later by the rejection of bacterial pleiomorphism (the thesis that all microbes could shift from their present form to any other and thus did not have constant effects or species characteristics) and the development of methods for the identification of numerous pathogens involved in disease and putrefaction (Drews 2000). The key method[17] for such rapid success was formalized by Robert Koch, whose 'postulates' of removing organisms from their complex communities and experimentally isolating the disease-causing process dominated microbiology for more than a century (despite the fact alternative 'mixed culture' and ecological approaches were available).

Koch's postulates emphasized two things: microbes as static individuals of single-cell types from which pure cultures could be developed, and tightly controlled uniform environments that were laboratory creations[18] (Penn and Dworkin 1976; Bull and Slater 1982a; Caldwell et al. 1997; Shapiro and Dworkin 1997). Both these emphases have skewed microbiology, and only in very recent decades has alternative work on bacteria as dynamically interacting components of multicellular systems in a diverse range of non-laboratory environments taken hold.

[17]Better microscopes and microscopy, chemical studies of metabolism, developmental investigations of eukaryotic microbes, and better classification systems all contributed to this period of success. See Drews (2000) for a comprehensive and succinct overview.

[18]Penn and Dworkin (1976, pp. 279–280) categorize these approaches as 'essentialist' (microbes as independent entities possessing intrinsic unchanging characteristics) in contrast to an 'interactive' or dynamic developmental understanding of microbes and microbial processes – an understanding available even in Koch's time.

Microbial biochemistry, genetics and molecular microbiology[19]

As bacteriology matured from medical and industrial applications into a biological discipline at the end of the nineteenth century, it increasingly used biochemical tools and analyses to understand the biological processes of bacteria and other microbes (Brown 1932; Summers 1991). The origins of modern biochemistry are, in fact, attributed to the isolation of fermentation enzymes from the microbe yeast in the late 1890s[20] (Kohler 1973; Manchester 2000). Biochemical investigation generated rapid growth of understanding of intracellular processes in bacteria and other microbes, but these insights were retained within the specialized domain of bacteriology and were of little interest to mainstream biology and genetics.

The transition from microbial biochemistry to molecular microbiology and microbial genetics took microbiology right into the centre of modern biology (Magasanik 1999). It was not until the 1940s that bacterial genetics was founded on the basis of the realization that bacteria have genetic material and that their study would enhance investigations of genotype–phenotype relations. This merger of biochemistry and genetics to study bacteria, viruses and unicellular eukaryotes was responsible for the greatest triumphs of molecular genetics in the second half of the twentieth century and had a profound impact on a range of other disciplines from evolutionary biology to epidemiology (Luria 1947; Brock 1990). Major breakthroughs gained via microbial analysis included many of the most famous insights into DNA, RNA and protein synthesis (e.g., Beadle and Tatum 1941; Luria and Delbrück 1943; Avery et al. 1944; Lederberg and Tatum 1946). In addition, the subsequent (1970s) development of recombinant DNA technology on the basis of knowledge of bacterial genetic systems generated a huge body of biological insight and biotechnological applications (Brock 1990).

Microbial sequencing and genomics

The experimental focus of molecular microbiology achieved enormous advances in microbiology and genetics, but it was painstaking work that continued to revolve around lab-cultured microbes. These approaches were still unable to produce data sufficient for a 'natural' classification system that would surpass the purely pragmatic one often considered unsatisfactory for a true microbial science (Stanier and Van Niel 1941; Stanier et al. 1957).

The advent of sequencing technology transformed microbiology's datasets and breadth of knowledge. The early sequencing revolution in microbiology

[19]Our description in this subsection of the period from the 1900s to the 1970s passes over the development of several other techniques and technologies in microbiology, perhaps most notably the electron microscope.

[20]For an alternative history, see Wainwright (2003).

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.[21] Zuckerkandl and Pauling had proposed that the evolutionary trees inferred from the comparison of genetic or protein sequence data from different organisms would map onto those inferred from traditional phenotypic characters and thus converge upon real macroevolutionary patterns (Pauling & Zuckerkandl 1963; Zuckerkandl and Pauling 1965). They posited that a molecular clock was ticking in these sequences in the form of accumulated mutations, and because of its regularity, the time of evolutionary divergence in sequences could be calculated (within a margin of error) and ancestral relationships much more firmly established. Early molecular work on the phylogenetic relationships between microbes used a variety of amino acid and nucleotide sequences, but Woese settled on small subunit ribosomal RNA (SSU rRNA) and rDNA sequences, particularly the 16S gene, as the best 'molecular chronometers' because of their ubiquity, highly conserved structure, functional constancy, predictable rates of variation in different regions, and practical ease of sequencing (Woese and Fox 1977; Fox et al. 1980).

Woese's discovery of the archaea dramatically transformed biology's basic classificatory framework of life from two fundamental domains or superkingdoms (prokaryotes and eukaryotes[22]) to three, and cast new light on the origins and subsequent differentiation of biological lineages. Although disputed by many taxonomists, especially those outside microbiology (e.g., Mayr 1998[23]), 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 – however reluctantly – as defaults (Olsen et al. 1986; Woese 1987; Woese et al. 1990).

The cumbersome methods and limited data of early microbial sequencing 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*[24] (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 (http://www.ncbi.nlm.nih.gov/

[21]Molecular sequences had been used to infer evolutionary relationships since the 1950s (Olsen et al. 1994), but Zuckerkandl and Pauling gave such efforts a much needed theoretical and analytical boost.

[22]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.

[23]'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.'

[24]Since renamed *Methanocaldococcus jannaschii*.

genomes). The comparative work done with these sequences has been enormous and has enabled an increasingly complex understanding of gene function and evolution (Brown 2001; Ward and Fraser 2005). Genomic insights have illuminated inquiries into the transition from prokaryotes to eukaryotes, indicated the minimal genome required to support cellular life, and tracked pathogenic diversity over the course of a disease and virulence mechanisms across a range of species (Schoolnik 2001; Ward and Fraser 2005). Simultaneously, however, genomic data pointed to phylogenetic contradictions between the 16S and other genes used as markers of evolutionary history. The inconsistent stories such markers tell challenge the practice of equating the evolutionary history of organisms with the history of molecules – a challenge we will outline and explore in the section below on lateral gene transfer.

Microbial ecology and environmental microbiology[25]

Ecological studies of microbes (historically not part of general ecology, but a subfield of microbiology) have been marginalized thoughout most of the history of microbiology by the pure culture paradigm and the lack of effective alternative methods (Brock 1966; Atlas and Bartha 1998; Costerton 2004). Early articulations of microbial ecology are attributed to Russian soil microbiologist, Sergei Winogradsky, and the founder of the famous Delft school of microbiology, M. W. Beijerinck, at the end of the nineteenth century. It was not until the late 1960s, however, with the availability of a range of new molecular methods and a revived ecological sensibility that microbial ecology began to flourish as a subfield that proclaimed the limitations of studying bacteria as isolated individuals in artificial environments (Brock 1987; Caldwell and Costerton 1996). These limitations were highlighted by the 'great plate count anomaly', which drew attention to the several orders of magnitude of discrepancy between microscopic cell counts of environmental samples and plate counts of bacteria cultured from those samples (Cutler and Crump 1935; Jannasch and Jones 1959; Staley and Konopka 1985). Once these discrepancies were no longer attributed to observed cells being 'non-viable', they led to estimates that as many as 99% of prokaryotes could not be observed or studied further because their culture evaded all available techniques[26] (Amann et al. 1995). Molecular microbial ecology is increasingly integrated with biogeochemical approaches that study microbial interactions with the chemistry and geology of ecosystems (Newman and Banfield 2002; Croal et al. 2004; Doney et al. 2004) and has been further enhanced by the development of imaging

[25]Microbial ecology is sometimes described as the 'basic' study of microbial interactions in environments, and environmental microbiology as their 'applied' study especially in relation to their effects on humans (Maier et al. 2000).

[26]These observations do not mean the abandonment of culturing, and many new culturing techniques are addressing microbes previously thought to be unculturable in order to supplement molecular and other ecological investigations (Joseph et al. 2003; Leadbetter 2003).

technologies that enable *in situ* observation at the cellular and subcellular level (Brehm-Stecher and Johnson 2004; Daims et al. 2006).

This environmental turn has also occurred within microbial genomics itself, which has extended its approach beyond laboratory cultures of microorganisms to DNA extracted directly from natural environments (Stahl et al. 1985; Olsen et al. 1986; Amann et al. 1995). 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 characteristics of the organisms (Staley and Gosink 1999; Brune and Riedrich 2000; DeLong and Pace 2001; Rodríguez-Valera 2002).

A potential remedy to these shortfalls lies in the development of metagenomics, an approach in which the DNA of entire microbial communities in their natural environments (the metagenome) is sequenced and screened and then further analysed in attempts to understand functional interactions and evolutionary relationships (Handelsman et al. 1998; DeLong 2002; Handelsman 2004; Riesenfeld et al. 2004; Rodríguez-Valera 2004).[27] These studies are not only discovering new genes and strains of prokaryotes and viruses, but are also revealing wholly unanticipated functions and mechanisms such as photobiology in oceanic bacteria (Béja et al. 2000; DeLong 2005) and the molecular complexities of symbiotic relationships (Kitano and Oda 2006). Metagenomics is still at a very early stage of constructing inventories of microbiodiversity, however, and it will need to integrate many other approaches in order to understand the complexity of microbial interactions in their diverse environments.

Prokaryotes as multicellular organisms

The tendency for other disciplines to ignore or marginalize microbes and microbiology may be because of assumptions that prokaryotes are simple separate cells that are behaviourally limited and the equivalent of evolutionary

[27]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). However, the full metagenome sequence of the most complex and diverse communities (especially in soils) is still beyond the reach of current technologies because of the size and complexity of the communal genome, which requires formidably high numbers of clones and sequence coverage to accurately represent the genetic composition of the community (Riesenfeld et al. 2004). In addition, the harsh process of extracting DNA from the soil sample breaks the DNA into very small fragments which may be unsuitable for studies that are interested in networks of genes rather than single genes (Handelsman et al. 1998; Daniel 2004).

fossils of life's primitive beginnings. A great deal of recent and older evidence can be marshalled in support of the very opposite conclusion: that bacteria are complexly organized multicellular entities with sophisticated and efficient behavioural repertoires (many elements of which are not available to multicellular eukaryotes) and that microbes are, in fact, the evolutionary sophisticates who exhibit far more capacity to adapt to dramatic environmental change than does multicellular eukaryotic life.

A growing group of microbiologists now argue that to study prokaryotes exclusively as unicellular organisms is highly misleading (Slater and Bull 1978; Caldwell and Costerton 1996; Shapiro 1998; Davey and O'Toole 2000; Kolenbrander 2000). Prokaryotes rarely live in isolation but in a variety of communal organizations that often include macrobes. Microbes engage in a range of associations with other organisms, some of which are competitive or parasitic, and others of which are commensalisms (benefiting one partner) or mutualisms that benefit all involved (Bull and Slater 1982b; Wimpenny 2000). Many of these may be loose or temporary, whereas others are more stable and obligate (e.g., endosymbiont or intracellular symbiotic relationships[28]).

Everyone may agree that there are intercellular relationships and loose communities, but the argument is about whether such interactions justify the postulation of multicellularity (e.g., Jefferson 2004). Traditional definitions of multicellularity emphasize task sharing by tissue differentiation and the permanent alteration of gene expression patterns, thereby excluding non-macrobial forms of cellular organization. However, a more encompassing definition is suggested by the molecular and cellular study of microbial communities. These communities exhibit well-defined cell organization that includes specialized cell-to-cell interactions, the suppression of cellular autonomy and competition, and cooperative behaviour that encompasses reproduction (Carlile 1980; Kaiser 2001; Keim et al. 2004).

By working together as functional units, microbes can effect a coordinated division of labour into zones of differentiated cell types that enable them access to a greater variety of energy sources, habitats, protection and other collective survival strategies (Gray 1997; Shapiro and Dworkin 1997; Crespi 2001; Webb et al. 2003). Many of these are activities that individual microbes are unable to accomplish and which are, in fact, often achieved at the expense of 'altruistic' individual microorganisms.[29] In the most common community structure of biofilms, individual cells usually show lower growth rates than do free-living

[28]Endosymbionts such as *Buchnera* in aphids and *Wolbachia* in numerous insects and other invertebrates are so integrated into their partner's cells that their genomes are greatly reduced, partly by loss and partly as genes are transferred from the symbiont's genome to the host's nucleus and the gene products are transported back to the endosymbiont (Andersson 2000; Douglas and Raven 2002). They may eventually become organelles of the host cell as did the proteobacteria that is now the mitochondrion and the cyanobacteria that became the chloroplast.

[29]Cheater controls are obvious objects of investigation to understand the fine-tuning of cooperation in prokaryote communities and there is some evidence to indicate they exist (Velicer 2003; Travisano and Velicer 2004), although this interpretation of the data is still somewhat controversial.

individuals (Kreft 2004). The 'suicidal' programmed cell death or autolysis (self-disintegration) of individual cells appears to directly benefit the group (Dworkin 1996; Lewis 2000; Ameisen 2002; Rice and Bayles 2003; Velicer 2003).[30] A great variety of communal strategies has been observed and experimented on in single-taxon populations, but the most common forms of complex cooperation are found in mixed (multi-taxa) consortia of prokaryotes and other microbes.[31] Their communal activities range from carrying out coordinated cascades of metabolic processes to the regulation of host-parasite interaction and environmental modification (Dworkin 1997; Shapiro 1998; Hooper et al. 1998; 2001; Kolenbrander 2000; Crespi 2001). Recent decades of studies of the collective behaviours involved in biofilm formation, chemotaxis, quorum sensing and genetic transfer give a great deal of support to the multicellular description of microbial communities.

Biofilms

Biofilms are the favoured lifestyle of most prokaryotes and are found in all microbial environments with surfaces, nutrients and water, from fast-flowing hot springs to catheters. They are often visible and may contain many millions of cells. Biofilms are constructed by microorganisms exuding and surrounding themselves with slimy biosynthetic polymers. Formation occurs in clear stages of adhesion, attachment, maturation and detachment (Costerton et al. 1995; Stoodley et al. 2002). Different environmental conditions influence a variety of biofilm architectures, and other materials and new species are incorporated into (or break away from) the biofilm as it develops. The prokaryotes in biofilms express genes in patterns that are very different from free-floating (planktonic) microbes, and gene expression in a biofilm changes at each stage of its development (Stoodley et al. 2002).

Living in a biofilm prevents the annihilation of bacterial communities in adverse conditions, even those of heavy and repeated antibiotic therapy[32] (Davey and O'Toole 2000; Wimpenny, 2000; Stewart and Costerton 2001). Biofilms enable close intercellular contact that involves the exchange of many different molecules and allows greater metabolic diversity, as in the multistage digestive processes carried out by prokaryotes in the bovine rumen, as well as genetic transmission between cells and the rapid acquisition of antibiotic-resistance or virulence genes (Watnick and Kolter 2000). Although biofilms have been studied intensively since the late 1970s, it is only in recent years that

[30]Even apparently non-cooperative acts of cannibalism appear to be beneficial for the group, because some components of the group are digesting other components in order to keep the whole alive (Engelberg-Kulka and Hazan 2003).

[31]One reason so few prokaryotes have been cultured may be because laboratory environments provide only nutrients and not signals from community members (Kaeberlein et al. 2002).

[32]Some researchers estimate that prokaryotes in biofilms have 1000 times more resistance to antibiotics than do planktonic prokaryotes (Davey and O'Toole 2000).

researchers have emphasized their biological aspects (over their physico-chemical) and begun to conceptualize biofilm formation as a multicellular developmental process (Davies 2000; O'Toole et al. 2000; Stewart and Costerton 2001). It is a more flexible form of development than metazoan development because although biofilm formation is directional, it is strongly influenced by environmental conditions, and is reversible and not locked into a rigid sequential process as is metazoan development (Parsek and Fuqua 2004; see Note 36).

Chemotaxis

Chemotaxis is the directed movement of cells to or away from chemical stimuli. First studied in the late nineteenth century, its molecular mechanisms were not understood until the late 1960s (Adler 1969; Eisenbach 2005). 'Bacterial' (including archaeal)[33] chemotaxis is achieved by a two-component signal transduction system that involves transmembrane receptors on the prokaryote cell. These respond to subtle changes in environmental chemicals and regulate the motor activity and type of movement, thereby altering the cell's direction (Falke et al. 1997). Moreover, chemotaxis is a social process in which prokaryotes are attracted by the chemicals secreted by neighbours. The assemblies they then form enable and enhance further social interactions associated with biofilm formation, communication and genetic exchange (Park et al. 2003).

A feedback methylation system (in which the methylation states of the receptors are modulated by enzymes affected by stimulus response) allows the cells to adapt to the initial stimulus. This process is frequently analogized to memory[34] because it allows cells to compare their present situation with the past and respond accordingly (Koshland 1979; Falke et al. 1997; Grebe and Stock 1998). The sophistication of these chemotaxis receptor systems has led some researchers to argue that they are 'nanobrains' – tiny organs with enormous computational power that use sensory information to control motor activity (Webre et al. 2003; Baker et al. 2005).

Quorum sensing

Quorum sensing is a form of communication-based cooperation that is often called 'chemical language' and analogized to hormonal communication between metazoan cells (Bassler 2002; Shiner et al. 2005). Quorum sensing can

[33]Different chemotaxis systems operate in a great variety of prokaryote and eukaryote cells. The most well-studied prokaryote system is that of *E. coli*, but *Bacillus subtilis* and *Rhodobacter sphaeroides* systems are also important as models (Wadhams and Armitage 2004). Eukaryote chemotaxis is often investigated in *Dictyostelium discoides* (cellular slime mould) and neutrophils (mammalian cells that track down infections) (Haastert and Derreotes 2004).

[34]For other instances of memory in prokaryotes and phage, see Casadésius and D'Ari (2002).

only be carried out in communities because it is population-density dependent. It involves the release of small signalling molecules (called 'autoinducers'), through which cells are able to assess population density.[35] When it is high and the molecules reach a threshold of concentration, they interact with proteins that regulate gene expression thereby activating collective behaviours from biofilm formation to the production of virulence or bioluminescence (Dunny and Winans 1999; Miller and Bassler 2001; Henke and Bassler 2004). The behaviour of individual cells thus reflects regulation at a multicellular level (Gray 1997) and indicates 'primordial social intelligence' (Ben Jacob et al. 2005). The communities in which quorum sensing operates include not only prokaryote species but also eukaryote hosts, where interactions may involve the bi-directional modulation of gene expression in host and commensals (Brown and Johnstone 2001; Federle and Bassler 2003; Shiner et al. 2005; Visick and Fuqua 2005).

Lateral gene transfer

The genome itself participates in the multicellular life of prokaryote communities through processes of genetic transfer between cells – perhaps the 'ultimate interaction' between organisms in communities (Dworkin 1997, p. 10; Shapiro 1997). Lateral or horizontal gene transfer (LGT or HGT) involves the transfer of diversely packaged genetic material from one organism to another most commonly by conjugation, transduction, or transformation. Conjugation is the transfer of DNA that involves cell-to-cell contact between organisms and the transfer of a mobile genetic element (a conjugative plasmid or transposon); transduction is the transport of DNA from one organism to another by bacteriophages; transformation is the direct uptake of free environmental DNA by a 'competent' organism into its genome (Ehlers 2000; Bushman 2002; Thomas and Nielsen 2005). Competence is an induced state of ability to bind, import and recombine free DNA (Solomon and Grossman 1996) – an ability that is at least partly regulated by extracellular chemical signals between organisms in communities (Dunny and Leonard 1997; Lee and Morrison 1999; Peterson et al. 2004).

The transfer of genetic material enables communities to adapt rapidly to changing environments (Reanney et al. 1982). Laterally acquired advantages include novel capacities with which to take over new environments, new metabolic functions, resistance to antibiotics, and increased pathogenic virulence (Levin and Bergstrom 2000; Ochman et al. 2000; Feil and Spratt 2001;

[35]There are three canonical quorum sensing systems or circuits, which are discussed in detail in Miller and Bassler (2001). Two are used for intra-species communication; the other for a wide range of interspecies communication (Federle and Bassler 2003). Many prokaryotes possess versions of more than one system (Henke and Bassler 2004). There is a little scepticism about whether quorum sensing is group communication or merely individual sensing of chemical diffusion (e.g.: Redfield 2002) but this is a minority interpretation.

Sonea and Mathieu 2001). The genes for the entire chemotaxis system, for example, were probably transferred as one unit between bacteria and archaea (Faguy and Jarrell 1999; Aravind et al. 2003). Current research indicates that genetic transfer by conjugation and transformation is much more frequent and efficient in biofilms than amongst planktonic bacteria (Hausner and Wuertz 1999; Molin and Tolker-Nielsen 2003; see Ehlers 2000 for methodological limitations of these studies). Genetic transfer and its mechanisms also appear to have positive effects on the development and stability of biofilms, meaning it is a communal activity that has both short-term lifestyle benefits as well as longer-term evolutionary benefits (Molin and Tolker-Nielsen 2003).

The capacity for lateral gene transfer in communities has many implications for evolutionary theory and taxonomic practice (discussed below), but the main point we are making here is that the 'one-organism one-genome' equation is insufficient to describe the genetic constitution of microbial communities. The concept of the metagenome is based on this extended understanding of a community genome as a resource that can be drawn on by the community organism – the metaorganism or superorganism. This genomic perspective backs up the notion of microbial communities as multicellular organisms.

The body of evidence above not only challenges the unicellular perspective in microbiology itself but also raises important issues for the philosophy of biology, especially in relation to how philosophers understand biological individuality, evolutionary transitions and processes, and the concept of species. We will examine each of these areas from the microbiological platform we built above, and outline some issues of major relevance to philosophers of biology.

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, as explained above, the notion of community in its various forms has also deeply informed the discipline's theory and research.

If communities are self-organizing entities that operate as functional units and are more than simple aggregations of individuals (Andrews 1998; Ben-Jacob et al. 2000; Kolenbrander 2000), they can only be excluded from multicellular status if the definition of multicellularity is closely based on knowledge of multicellular eukaryotes. Broader definitions (mentioned above) are able to include groups of interacting microbes, of one or many taxa, including sometimes eukaryote hosts (Dworkin 1997). 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.

Philosophers may want to ask some basic questions about the ontological status of microbial communities, particularly whether the community organism is more fundamental than the individual organism. 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 (Looijen 1998; Parker 2004). Communities of plants, for example, do not typically appear to 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 rather than as biological individuals (Underwood 1996; Collins 2003). The notion that communities might have emergent properties that individuals do not is explicitly rejected by many ecologists (e.g., Underwood 1996). This 'boundary problem' for communities of plants and animals is presumed to be even worse for microbes, which are generally considered to be globally distributed and environmental will-o'-the-wisps (Finlay and Clarke 1999).

A first response to these doubts might be that clear boundaries are not necessarily connected to ontological fundamentality. 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. Second, the biofilms that are the preferred lifestyle of prokaryotes make possible their study as bounded multicellular entities as well as contradicting common conceptions of bacteria as free-floating individuals in occasional and highly impermanent contact. Finally, there is a large body of empirical work which challenges standard views of boundaries because it reverses expectations about organismal integrity and microbial ubiquity. In regard to the former, the omnipresence of genetic exchange in microbial communities shows organism boundaries to be much more permeable than might have been thought. For the latter, 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 bacteria and archaea in hot springs and soils, for example, do actually have geographic limits at the strain level (Cho and Tiedje 2000; Whitaker et al. 2003; Papke and Ward 2004).

Communities may not possess the level of physiological integrity that individual (monogenomic) organisms do, but the recent research that we have outlined clearly indicates that they are much more than just individuals who happen to have blundered together. It seems more promising to conceptualize microbial communities as individuals with somewhat indeterminate boundaries that have some 'un-organism-like properties' (McShea 2004) while still possessing many organismal (or proto-organismal) characteristics. 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 avenues of research mentioned above concerned with understanding the multicellularity of bacterial communities appear to demonstrate such 'downward' causation, and at the least provide strong reasons for pursuing this issue further.

Evolution

Evolution has, for the most part, been about microbes, and many of the most fundamental evolutionary questions revolve around unicellular life: how life began, how prokaryotes evolved to eukaryotes, and how transitions from unicellular to multicellular life were accomplished. The philosophy of biology is, of course, interested in these issues but primarily as a background to its evolutionary focus on multicellular organisms. The neglect of microbes can be particularly striking in one of the most exciting topics in philosophy of evolution, evolutionary developmental biology or 'evo-devo'. For example, Robert (2004, p. 34), in a pioneering philosophical treatment of ontogeny, 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 macrobial sense, develop at all,[36] it would appear that on this view they are not biological systems and apparently could not have evolved. Of course, as we have been arguing, it might turn out that individual microbes are not the best way to understand microbial organization and development, and it may be that only as communities could they have evolved. But it is doubtful whether

[36]Prokaryote development has been intensively researched for over two decades (Figge and Gober 2003; Kroos and Maddock 2003) but it is about something very different from eukaryote multicellular development, which is how development is almost invariably conceived outside microbiology. Eukaryote development involves the differentiation of cell lineages leading to tissues with specialized physiological functions, morphological complexity and growth, with sexual reproduction as the main source of genetic diversity. Prokaryote development is primarily environmentally initiated (although it can also be an internally cued stage in a cell division cycle, such as in *Caulobacter*), and is usually uncoupled from single-cell growth. Genetic diversity is obtained via a number of other strategies (see above). A commonly used definition of prokaryote development is 'a substantial change in form as well as function in the life cycle of the cell' (Dworkin 1985, p. 3), which may take either unicellular or multicellular forms (as in myxobacteria aggregations). There are four main categories or cycles of prokaryote development: resting cells, complementary cell types, dispersal cells, and symbiotic development (Shimkets and Brun 2000). Individual cells can still leave developing multicellular units and enjoy their own singular fate rather than the developmental fate of the multicellular group (Shimkets 1999). Prokaryote development therefore involves different organizational strategies, different selective pressures, and much more genetic and biological diversity than does eukaryotic multicellular development (Shimkets and Brun 2000). There are also some phenomena common to both, however, and these include self-recognition, spatially directed growth, specialized cell differentiation, intercellular signalling and programmed cell death (Shimkets 1999).

communities have exactly the kind of developmental properties that the eukaryotic multicellular vision requires, and it is certain that Robert did not intend to describe the development of prokaryote communities. Surely it reflects an oversight, but one we think is very telling of the tendency for philosophy of biology to focus exclusively on macrobes. It also nicely illustrates how evolutionary microbiology can enrich and challenge standard evolutionary theory.

Units of selection and evolutionary transitions

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 at group and other levels (Brandon and Burian 1984; Sober and Wilson 1994; Wilson 1997). Although considerable conceptual progress has been made over the last two decades (Brandon 1999; Lloyd 2000; Okasha 2003), prokaryote communities have hardly ever been used as illustrations or objects of analysis in the debate.[37] One of the obvious questions the discussion of community function raises is whether these apparently coevolved relationships and community-level properties are selected for, or whether their existence can be fully accounted for by selection at the individual gene/organism level (Collins 2003; Whitham et al. 2003). Can such entities as prokaryote communities be conceived of as units of selection? There is experimental evidence that supports group selection in prokaryote communities (e.g., Queller 2004).[38] Is there competing selection of individual cells and genes that threatens the cooperation achieved at the community level? If we accept the arguments for microbial communities as biological individuals, then it is a plausible speculation that systems involving commensal microbes and sometimes macrobes could be considered to be *the* standard unit of selection. Community-level accounts of selection may even provide the key to identifying the mechanisms that allowed a hierarchy of biological organization to evolve in the first place (Okasha 2004, 2003).

One of the great benefits of attention to microbes is that it draws attention to the problem, easily overlooked when the transition to multicellularity is interpreted as self-evident progress, of *why* multicellularity evolved at all. Explanations of the evolution of multicellularity tend to take it for granted that eukaryotic multicellularity is obviously superior, so the discussion tends to be about *how* it evolved. For the multicellular organism to have become an

[37]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 1994). 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.

[38]Queller (2004) reports on the experimental results of Griffin et al. (2004), who find that the best interpretation of social behaviour in *Pseudomonas aeruginosa* is group selection, not kin selection.

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, 1997b) 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 still suffers from a residually 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 or the specialization of reproductive cells grounds one very interesting form of cellular cooperation, but perhaps we should avoid thinking of it as the only possible form. If there is something incoherent about the idea of an organism reproducing through the independent reproduction and subsequent reintegration of its parts, it is an incoherence that needs to be demonstrated.

The preceding point can be seen as part of the broader project of rethinking much more generally the possibility for aggregation of cells into more complex structures. We are inclined to speculate that macrobial multicellularity (like organelles in eukaryote microbes) is just a frozen, less flexible, obligate analogue of bacterial multicellularity. Prokaryote cell differentiations can dedifferentiate whereas metazoan multicellularity is irreversible. In eukaryote multicellularity, for example, aerobic metabolism is essential because this form of multicellularity has high energy demands that cannot be met by anaerobic means (Fenchel 1996). Prokaryote multicellularity, however, is an energy-efficient form and metabolic diversity is not sacrificed. The eukaryote 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. Microbes have a proven track record of living in a world devoid of eukaryotes, but multicellular eukaryotes are unlikely to be able to manage in a microbeless ecosphere.

In many ways, microbial communities have experienced a great deal more evolutionary and ecological success than macrobes. 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[39] (unless, indeed, we should see

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

macrobes in a neo-Dawkinsian way, as primarily vehicles for the billions of microbes that live in the many niches macrobes provide, designed to transport them to especially large and attractive energy resources).

At any rate, we need to resist the temptation to see microbes as primitive precursors of microbes and the transition to multicellularity as representing unambiguous progress. Rather, we must 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 is also, 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).[40]

Evolutionary process and pattern

As important as these questions about major evolutionary transitions is the need to reflect on the mechanisms by which microbial communities adapt and evolve. The philosophy of evolutionary biology must take account of the rapidly growing body of work in microbial phylogeny on horizontal or lateral gene transfer. The capacity for resource exchange that LGT allows 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).

The findings of comparative evolutionary genomics have raised enormous problems for the dominant eukaryo-centric paradigm of vertical inheritance and mutation-driven species divisions that give rise to a single tree of life (Doolittle 2002, 1999; Stahl and Tiedje 2002; Gogarten and Townsend 2005; O'Malley and Boucher 2005). 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) by revealing huge amounts of atypical DNA in numerous genomes. Many genomic sequences do not match organismal or species patterns due to the complex histories of gene exchange. Frequent transfers result in mosaic

[40]See Martin and Russell (2003) for an evaluation of competing hypotheses on eukaryote origins, and McFall-Ngai (2001) for an argument that symbiosis with microbial communities has been a key factor throughout the evolution of multicellular organisms.

genomes which consist of genetic contributions from many sources, even phylogenetically distant ones (Koonin et al. 2001; Doolittle et al. 2003; Lawrence and Hendrickson 2003). This lack of a unilinear history to genomes has inspired a number of methods that attempt to capture not only vertical lines of descent (as bifurcating tree branches) but also the web-like complexity of lateral movement between lineages[41] (e.g., Huson 1998; Bryant and Moulton 2004).

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 (Staley 1997; Stahl and Tiedje 2002; Lawrence 2002; Weinbauer and Rassoulzadegan 2004; Myers et al. 2006). It seems likely that the biologically significant loss in a microbial context would be something like a metabolic capacity rather than a particular microbial strain. But given the possibility of a wide distribution of genomic resources underlying these capacities, such extinction may be an improbable event. If so, then extinction, which plays a major role in standard models of macroevolution, is irrelevant for theorizing the evolution of microbes.

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. 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). 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 communities. 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 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 aquatic bacteria, say, is another matter. If not, either microbial evolution is

[41]The debate continues about whether the vertical lines in molecular phylogenies of prokaryotes are overwhelmed by lateral lines. Some recent studies have managed to recover an approximate 16 S-defined tree structure from very large datasets (e.g.: Beiko et al. 2005).

limited to more peripheral, isolated environments or, more likely, we will need to expand on traditional macrobial models in search of an adequate understanding of microbial 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.

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., Hull 1987a; Sterelny 1999; 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 often associated with eukaryotic species and classification schemes (Roselló-Mora and Amann 2001). There is, of course, controversy over how sharp the species boundary is even in eukaryotes but to whatever extent it is a problem there, it is considerably worse in prokaryotes (Dupré 2002).

The early history of microbial classification is a struggle for the specificity of bacteria and the recognition that groups have inherent characteristics that distinguish them from other putative species groups (Cohn 1875, in Drews 2000). 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 (Lan and Reeves 2000; Doolittle 2002; Konstantinidis and Tiedje 2004). Although the biological species concept (BSC) has never found much purchase in microbial systematics because of its exclusion of asexual reproduction and difficulties in coping with gene transfer between evolutionarily distant lineages (Maynard Smith 1995; Cohan 2002; Dupré 2002), there is an active debate between microbiologists about what constitutes an appropriate evolutionary or phylogenetic definition (Roselló-Mora and Amann 2001). In its simplest form, this simply means species are defined by common ancestry.

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 above, the role of 16S rRNA gene sequence as the ideal phylogenetic marker has been undermined by conflicting genomic evidence, which has also damaged more generally the idea of a single true marker for microorganismal 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 (Palys et al. 1997; Cohan 2002; 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, and these must be constantly revised in light of new evidence and emerging inconsistencies.

Popular operational measures reflect the mixture of concepts and conceptual problems at work in microbial systematics. The currently predominant 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 (Dijkshoorn et al. 2000; Roselló-Mora and Amann 2001). This crude measure of genomic distance is commonly considered equivalent to 97% rDNA 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 (Lan and Reeves 2000; Cohan 2002). Apart from the fact that 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 considered adequate representatives of a species history (Palys et al. 1997; Boucher et al. 2001; Lan and Reeves 2001). 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 physiologically diverse strains (Staley and Gosink 1999; Kämpfer and Rosselló-Mora 2004).

An influential proposal designed to overcome these problems is the quasi-official (American Society of Microbiology) species definition (Vandamme et al. 1996; Stackebrandt et al. 2002). It combines genomic, phylogenetic and phenotypic approaches into a pragmatic and 'phylophenetic' (or 'polyphasic') taxonomic framework in which a species is '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, p. 59). In practice, however, any such practical species measure is still anchored phylogenetically by the 16S rRNA gene (Dijkshoorn et al. 2000; Young 2001) which is seen as a proxy for natural units and their boundaries, and helps overcome the discomfort of many microbial systematists with 'non-natural' classification concepts and methods (e.g., Ward 1998; Coenye et al. 2005).

Another operational measure with the aim of natural classification uses the concept of a 'core' genome. Although there were earlier hopes of finding a phylogenetically definitive universal core of genes common to all prokaryotes, current measures focus on pools of genes that determine 'properties characteristic of all members of a species' (metabolic, regulatory and cell-division genes) and are seldom transferred (Lan and Reeves 2000). Because there is presumed to be a barrier to the interspecific recombination of core genes, they reveal the evolutionary history of the species (Wertz et al. 2003). Core genes are contrasted to more variable 'auxiliary' genes which often enable niche adaptation but are unreliable as species indicators.[42] There is still, however, great difficulty in finding genes that provide core conserved functions but are not transferred (Boucher et al. 2001; Doolittle 2005; Saunders et al. 2005) and different patterns of variability and stability in genomes of different species may require a range of species-genomes concepts. The idea of a core genome may be capable of providing a definition of species, but is unlikely to ground a fully phylogenetic taxonomy given the prevalence of lateral gene transfer over deep time.

If, as is strongly suggested by the several lines of research outlined 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 are doomed to failure. Microbiologists should be well prepared for the discovery that species genomes or phylotypes (a taxon defined by a particular gene marker) 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.[43]

Many further questions remain in this area. Is there potential for a taxonomy of communities or community lineages, or do these entities have limited 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 communal genome as a dynamic community resource further undermine the notion of stable species boundaries, what are the implications for how we understand biodiversity?

[42]Together, these categories of genes make up the 'pan-genome' of a species, sometimes called the 'clade-specific metagenome' (Lawrence and Hendrickson 2005; Medini et al. 2005).

[43]As noted above, there is a question of how true this is for eukaryotes, but the problems for prokaryotes are surely more extreme.

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, 2005), mostly because of methodological and technical limitations. Microbiologists have long known that their understanding of microbial diversity has been restricted both by technology and by a health- or agriculture-based bias towards pathogens. Microbes' enormous diversity of habitats, metabolic versatility and physiological adaptability are still only beginning to be understood. Genomics-driven estimates have risen to as many as 10^7–10^{12} prokaryote 'species' (Dykhuizen 1998),[44] 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[45] (Kämpfer and Rosselló-Moro 2004).

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 anticipated for metagenomics is the contribution to a broader and deeper understanding of microbial diversity.

At present, broad studies of microbiodiversity are largely occupied by cataloguing exercises, but as the research deepens to include multilevel interactions and processes rather than things, the object of study could become biodiversity in the extended functional sense of how microorganisms are involved in ecosystem processes such as resource use, decomposition and nutrient cycling (Finlay et al. 1997; Loreau et al. 2001). 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 2004). They also need to incorporate explanations of 'the tempo, mode and mechanisms of genome evolution and diversification' in relation to higher-order biological and ecological processes (DeLong 2004; Falkowski and de Vargas 2004) and obviously the findings of biogeographic patterns in the distribution of prokaryotes and other microbes (Martiny et al. 2006; see above) will be part of this analysis.

Clearly, these are not straightforward research programmes that will give simple answers about biodiversity, but they are aspirations towards

[44]Rough estimates of virus species posit ten times more of them than prokaryote species (Rohwer 2003).

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

understanding complex phenomena for which technology and tools of analysis are beginning to develop. 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). It remains to be seen whether we *should* be much concerned about microbial conservation. Our remarks above about extinction raise the question of whether there is any serious risk to be evaluated. However, given the fundamental role of microbes in all life, it would be good to know how microbial diversity is affected by environmental changes already profoundly affecting macrobial biodiversity. Philosophical analysis could make important contributions to framing the questions that need to be asked.

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. The range of diverse and interconnected microbiological perspectives that we have outlined above 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.

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 (difficulties in coming up with a natural classification system and measures of diversity) is an implausible answer, as it might just as easily have stimulated philosophical scrutiny. 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 and still unchallenged attributions of status to zoology and animals (over botany and plants as well)? 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. But philosophers have shown no reluctance to get involved in debates about the molecular minutiae of other biological findings, so this explanation is not compelling either. Similarly one might point to the rapid development of techniques and theoretical frameworks in microbiology as inhibiting factors, but this rapidity would not distinguish it from various other biological subfields, especially in molecular biology, with which philosophers have been quite willing to keep up to date.

Some scientists perceive 'an unspoken philosophy of "genomic supremacy"' (Relman and Falkow 2001, p. 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 – whose genomes are up to 200 times larger than those of humans (Gregory 2001) – 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 need to propose a broader view of human supremacy (Paabo 2001) and comparative genomics is more likely to challenge such a notion than to support it.

Taking this explanation in terms 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 1962; 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, delving even briefly into the recent microbiological literature might provide just enough of a conceptual kick to initiate a wider range of thinking in the philosophy of biology and perhaps even stimulate a philosophy of microbiology.

Acknowledgements

Many thanks to our anonymous referee for very helpful advice and detailed comments; to Staffan Müller-Wille, Jane Calvert and Jim Byrne for feedback; and also to the audiences at the first International Biohumanties Conference (Queensland, 2005) and the International Society for the History, Philosophy and Social Studies of Biology conference (Guelph, 2005). We gratefully acknowledge research support from the UK Economic and Social Research Council (ESRC), the Arts and Humanities Research Council (AHRC), and Overseas Conference Funding from the British Academy. The research in this paper was part of the programme of the ESRC Research Centre for Genomics in Society (Egenis).

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