

From endosymbiosis to holobionts: evaluating a conceptual legacy

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Abstract

In her influential 1967 paper, Lynn Margulis synthesized a range of data to support the idea of endosymbiosis. Building on the success of this work, she applied the same methodology to promote the role of symbiosis more generally in evolution. As part of this broader project, she coined the term 'holobiont' to refer to a unified entity of symbiont and host. This concept is now applied with great gusto in microbiome research, and often implies not just a physiological unit but also various senses of an evolving system. My analysis will track how Margulis came to propose the term, its current use in microbiome research, and how those applications link back to Margulis. I then evaluate what contemporary use says about Margulis's legacy for microbiome research.

Keywords: Margulis; holism; homeostasis; microbiome; microbiota

1. Introduction

There is more to the legacy of Lynn Margulis's 1967 'On the Origin of Mitosing Cells' than the ongoing accumulation of evidence for endosymbiosis. Margulis used her success with this paper to issue methodological directives and challenge evolutionary theory. Methodologically she argued that biological systems are necessarily combinations of organisms, and thus need to be researched as such. This directive was of particular relevance for investigating evolutionary diversification points, which she believed were due to previously separate organisms forming permanent fusions. Only the bare outlines of this position were apparent in 1967, but it did not take Margulis long to flesh it out. As she did, over the next two decades in particular, she created a platform on which contemporary research finds some footing. Although symbiosis research once focused on specific microbe-host relationships, it has now become common to conduct molecular analyses of whole microbial communities and their relationships with hosts. In some of this recent research, there are affinities with Margulis's ideas. By casting some philosophical light on her work, I show how its problems have been inherited by contemporary researchers.

My plan is to trace in a critical and normative way the development of Margulis's basic claims as they grew from a somewhat open view in 1967 into a more dogmatic evolutionary and methodological stance. In 1990, at the peak of her elaborations of this stance, she coined the term 'holobiont' to capture the intimacy of microbe-host

relationships (Margulis, 1990b). I will show how the general conceptual machinery in which this term was embedded has proved to be very attractive for a subgroup of microbiome researchers, especially from the mid-2000s onwards.¹ Contemporary holobiont researchers study not just microbe-host pairs, but entire communities within various animal hosts (with occasional notice of plants). Such communities may involve thousands of species doing a great variety of different activities. Just as Margulis did, these researchers work with a loose conceptual version of evolutionary theory instead of one driven by population genetics. Following Margulis, they view multispecies 'systems' as their unit of conceptualization rather than single lineages. This occurs even though microbiome research relies heavily on DNA snapshots of such communities, and Margulis herself greatly doubted molecule-driven analysis. Disputes are raging over contemporary holobiont thinking about such communities, especially when evolutionary claims about these putative systems are asserted. I will outline the variety of problems associated with such claims, and then assess the potential future of Margulis's legacy for microbiome approaches to microbial communities and their evolution.

2. Margulis's basic ideas and how they developed

In 1967 as Margulis (Sagan) outlined how diverse evidence pointed to the endosymbiotic origins of mitochondria and chloroplasts, she stated a general consequence: 'If this theory [endosymbiosis] is correct all eukaryotic cells must be seen as multi-genomed systems' (Sagan, 1967, p. 271). In the same paper she discussed 'recent advances in molecular biology' (p. 249) – including knowledge about molecular homologies, mutational steps and DNA base ratios – as crucial to understanding phylogenetic relationships between microorganisms, including those ancestral to mitochondria and chloroplasts. Very soon afterwards, however, she began to resist molecular biology's reach into biology on the grounds of the inability of molecular analysis to capture genuinely organismal biology. Molecular phylogenies, which ultimately provided the confirmation of her revised account of endosymbiosis (Gray and Doolittle, 1982), were dismissed as 'intrinsically limited' (Margulis, 1992, p. 42). According to Margulis, molecular phylogenies were neither 'total' histories of organisms nor able to encompass the reticulation that represented endosymbiosis (the integration of one cell inside another). As she began to advocate strong evolutionary views that focused on fusions of organisms, Margulis insisted that Darwin's view – especially as formulated by the modern synthesis – was too limited. Gradualistic evolution had been her sole evolutionary target in the 1967 paper (Sagan, 1967, p. 272), but it became just one aspect of a larger complaint as she expanded her criticisms over the next few years.

Margulis believed the most basic problem for Darwin and the neo-Darwinians was the lack of a compelling account of the origins of new lineages and biological innovations.

¹ In much of the following discussion, I focus on the smaller group of microbiome researchers who use the concept of holobiont (and hologenome). I am not including all microbiome research and researchers in this analysis, except in places where I make this clear (i.e., in the discussion of homeostasis, Section 4.3).

‘Although the title of Darwin’s great book (1859) promised a solution to the problem of the origins of biological diversity ... his work far better explained the *maintenance of species by natural selection* than their first appearance’ (1993a, p. 121, emphasis added).

Her view of speciation did not accept that the mechanisms proposed by the modern synthesis could account for the origin of diversity.

‘I assert that the gradual accumulation of random mutations is insufficient to explain speciation. Rather, speciation occurs mainly by symbiogenesis, of course supplemented by DNA mutations’ (Margulis, 1993b, p. 9).

She argued that symbiosis-driven speciation was sudden and brought about large structural and functional changes. It could only be caused, she thought, ‘by the acquisition of hereditary genomes through protracted physically contiguous associations, primarily with microorganisms’ (Margulis, 1993a, pp. 121-122).

Symbiogenesis thus explained what appeared to Margulis and a few other evolutionary thinkers as a punctuated record of evolutionary change. Importantly, she argued that symbiogenetic explanations need not be based on theoretical claims that foreground cooperation rather than conflict (an emphasis in previous anti-Darwinism). As she clarified her views later in her career, after some early flirtation with anti-competition thinking, she said:

‘Symbiosis has nothing to do with “co-operation” or “mutual benefit” ... Symbiosis simply refers to the long-term physical association between members of different species ... Symbiogenesis is the evolutionary consequence of symbiosis. Because of long-term symbiosis in some cases new forms appear’ (Margulis, 2001, p. 59).

But crucially for Margulis’s account of speciation and the scope of her evolutionary concepts, she viewed the sudden symbiogenetic creation of species as exclusively a property of eukaryotic evolution, not prokaryotic:

‘The phenomenon of speciation, even though the words “species” and “speciation” are used as if they meant the same phenomenon, is a process only of eukaryotes. The jumps, the saltations, from prokaryotic components to composite “individuals” is a property of all eukaryotes. All have evolved symbiogenetically. Symbiogenesis is fundamental to all eukaryotic taxa from species to phyla ... Gradualism, the gradual accumulation of random mutations, ironically is more characteristic of prokaryotes. No one has ever shown, in the laboratory, field or fossil record, the veracity of that the Neo-Darwinist mantra [for eukaryotes]. That “gradual accumulation of random mutations” results in a passage from one to another, new and different, [eukaryotic] species has never been documented’ (Margulis, 2010a, p. 1538).

According to Margulis, prokaryotes – because of lateral gene transfer – either did not form species or formed only one single ‘worldwide’ species of equal-opportunity gene exchange (2010a, p. 1538). In other words, prokaryotes did not undergo or exhibit macroevolution, only microevolution.²

This non-gradual and major contribution to eukaryote macroevolution was, therefore, where Margulis saw the main challenge to the modern synthesis, and the reason why symbiosis is so conceptually important (despite many arguments that the

² This is an extraordinarily controversial claim, but not one I will address further in this paper.

modern synthesis can accommodate it – see O'Malley, 2015). Her differences with the modern synthesis fall into both process and pattern aspects of evolution: the heritable fusion of genomes (as in exemplar cases of endosymbiosis) is the process that produces the non-gradual origin of eukaryotic species and thus new phylogenetic patterns. Symbiogenesis, she argued, has the potential to bring about 'the appearance of new tissues, new organs, physiologies or other new features that result from protracted symbiotic association' (Margulis, 2004, p. 172). All of these innovations, according to Margulis, could produce new eukaryotic species and thus explain their origins (unlike Darwinian and neo-Darwinian evolutionary theory). She elaborated on these views with increasing stridency as each decade went by after her original JTB paper. Not long before the end of her life she predicted that

'The widely touted but undocumented explanation of the origin of evolutionary novelty by "gradual accumulation of random mutations" will be considered an erroneous early 20th century hunch proffered primarily by Englishmen, North Americans and other anglophones. They (Neodarwinist "explanations") will be replaced by the details of symbiogenesis' (Margulis, 2010a, p. 1525).

Her death has not brought an end to such claims about the modern synthesis. A broad form of this reasoning has been taken up and given new life by contemporary holobiont researchers.

3. Margulis and microbiome research

There are three main connections made by some of today's microbiome researchers – specifically, the subgroup making holobiont claims – to Margulis's general ideas. These links begin with her views about holobionts and their evolution, which lead to her methodological preferences (which I will call simply holism), and culminate in her organizational account of biological systems as homeostatic and autopoietic individuals.

3.1 Holobionts

In 1990, Margulis coined the term 'holobiont' as she argued for analogies between meiotic sex and symbiosis. In her view, both processes require partner recognition, mergers, the creation, integration and maintenance of new individuals, and ultimately, their dissociation (Margulis, 1990b, p. 676). She thought meiotic sex might be 'more ritualized' (i.e., following a specified series of pathways), but that this difference did not detract from the similarity of the two processes. Symbiosis, when it became symbiogenesis, was at least as responsible as sexual recombination for 'morphogenetic innovation and speciation' (Margulis, 1990b, p. 676). However, in drawing these analogies, Margulis did not develop at all what sorts of biological partnerships qualified as holobionts. Her definition of holobionts in 1991 was similarly unelaborated: 'holobiont: symbiont compound of recognizable bionts' (Margulis, 1991, p. 2). In later work, Margulis qualified such entities as 'integrated hereditary symbionts' (1992, p. 42), by which she meant primarily the mergers that had produced the organelles of mitochondrion and chloroplast (plus the eukaryote

flagellum³). She then extended this term to other obligatory symbioses as her work encompassed more prokaryotic examples. Recall, however, that her unorthodox views about evolution targeted eukaryotes only, and prokaryotes featured only where they contributed to eukaryote evolution.

Margulis's neologism of holobiont has taken on a new life in today's microbiome research. Microbiomes are the molecular components (particularly DNA) of microbiota, which itself is shorthand for the entire microbial community in a given environmental sample. Sequencing technology has revealed the molecular makeup of such communities, and the ubiquity, diversity and – in some cases – functional importance of microbes in a wide range of habitats. Initially, in the early 2000s, microbiome research achieved its successes in ocean and soil microbial ecology, but more recently major advances have been made by focusing on microbiota in humans and other animal hosts. Although many researchers in this rapidly expanding area are content to use the words 'microbiota' and 'microbiome' very casually, a small subset of scientists are promoting the conceptually loaded terminology of holobionts (host and microbiota) and hologenomes (host genome and microbiome).⁴ This terminology both draws on and extends Margulis's original use, in ways that complicate her legacy.

Initially, any evolutionary biologist in the 1990s who talked about holobionts also used the term in the more restricted sense Margulis intended, of specified evolutionarily persistent partnerships – particularly vertically inherited endosymbioses (e.g., Mindell, 1992). In the early 2000s, however, a group of coral researchers found themselves without a word that would cover the variety of microorganisms that came together with the animal host to make corals. Forest Rohwer and colleagues (2002) explained how microbial communities (prokaryotic and eukaryotic) formed 'species-specific associations' (p. 6) in the formation of coral holobionts. But notably, this revival of the word by Rohwer and other coral researchers did not argue that multispecies entities were evolutionary units of selection.

For many of the rest of today's holobiont advocates, however, there is a rapid conceptual slide from microbes and host being physiologically connected to microbes and host being a single evolving unit on which evolution can act. As this conflation of physiological and evolutionary notions of holobiont was further elaborated in the 2000s, it became entangled in many of Margulis's evolutionary commitments, and indeed, strengthened them. Lamarckian claims came to the foreground, especially when the cognate term 'hologenome' is used (e.g., Rosenberg et al. 2009). Any such claims are not, however, closely connected to

³ The eukaryote flagellum is not related to bacterial or archaeal flagella. For this reason Margulis insisted it should have a different name. She endorsed a term originally proposed by Russian biologists, 'undulipodium', but it was not taken up popularly (see Archibald, 2014). Her account of the evolution of the flagellum has not been broadly accepted and nor is it likely to be, as Margulis herself noted as early as 1975.

⁴ As is occurring in standard microbiome research, where microbiome is often used to mean microbiota, so too is 'hologenome' sometimes used for the community organism (holobiont).

historical Lamarckian ideas.⁵ Holobiont researchers simply mean that characteristics acquired from environmental sources (e.g., microbes) might somehow be inherited by the host. This may even be vertically if bacteria-eukaryote gene transfers or endosymbioses happen (Bordenstein and Theis, 2015; Zilber-Rosenberg and Rosenberg, 2008).

Much more significant for evolutionary theory than any casual mention of Lamarck is the contemporary holobiont position that the unit of selection is the host plus all its microbial symbionts. Although Margulis insisted there is no single-genome eukaryotic species, she focused only on specified relationships with long entrenched evolutionary histories (e.g., the mitochondria and chloroplasts in eukaryotic cells; exosymbionts forming *Mixotricha*; lichens; 'cyclical' associations of organisms such as squid and their luminescing *Vibrio fischeri*; *Wolbachia*-arthropod symbioses). Only some of these had undergone lineage fusion. Significantly, she did not extend her evolutionary thoughts on holobionts to all members of, for example, an animal gut community, even though microbiome analyses and their implications were well developed before her death in 2011. However, just as for contemporary versions of the holobiont concept, vertical inheritance of symbionts was apparently unnecessary to some of Margulis's holobionts (e.g., her 'cyclical symbioses'). I will return below to the heredity issues where they arise in microbiome research (Section 4.1), after looking first at two other aspects of Margulis's work and their connections to holobionts.

3.2 Holism

Although Margulis seemed quite open to molecular sequence data in 1967, her attitude became narrower and more hostile as her evolutionary ideas crystallized. This may partly be explained by the fact that many molecular findings did not support her more extensive ideas about endosymbiotic origins (e.g., of the eukaryotic flagella/cilium, which she thought led to the generation of the nucleus). She began to emphasize that her own research and microbiology in general should be understood as a branch of microscopy, which 'had no equal' methodologically (Margulis, 1975, p. 267).

'I have tried to resist the confusing pressures to fragment and technologize from which we all suffer in today's information glut. [My] work itself plunges ahead against the disdain, dismissal and ignorance of specialists and rejection by granting agencies ... [I write for those] who love the field more than the laboratory and refuse to apologize for their direct sensory interest in the natural world and its history' (Margulis, 1993b, p. 10).

⁵ Margulis's one mention of 'Lamarckian' views of evolution in her 1967 paper appears to be agnostic (Sagan, 1967, p. 269), but she became more pro-Lamarckian as she elaborated her evolutionary framework. She viewed symbiogenesis as demonstrating both Darwinian and neo-Lamarckian processes (e.g., 1990b, p. 677; however, see Margulis, 1975 for an exclusively Darwinian account of endosymbiotic evolution). This was because mitochondria, plastids, and 'other organelles' were acquired (during the original endosymbiotic event) and subsequently inherited. But as Ernst Mayr observed, in his foreword to Margulis and Sagan's *Acquiring Genomes*, endosymbiosis was not properly Lamarckian because it was about 'the inheritance of incorporated parts of genomes' rather than Lamarck's focus on 'inheritance of modified phenotypes' (Mayr, 2002, p. xiii). Because loose Lamarckian claims were not a major conceptual element of Margulis's critique of neo-Darwinian evolution, I have not focused on them, nor on their reappearance in contemporary holobiont research.

In other words, according to Margulis, proper classification and evolutionary biology could only be done on the basis of 'whole-organism biology' (Margulis, 1996, p. 1074; see also Ruse, 2013). This is a particular kind of holism, often known as 'organicism', in which organism-level descriptions and explanations are seen as having a special mandate. Disciplinarily, Margulis studied protists (eukaryotic microbes) before she broadened her work to discuss prokaryotic microorganisms that had relationships with eukaryotic hosts. Protists, obviously, have more morphology than most Bacteria and Archaea, plus many observable behaviours, and it used not to be uncommon for protistologists to resist molecular methods or at least to see them as merely supplementary to the cell biological research allowed by microscopy.

No single gene, Margulis argued with increasing emphasis (e.g., Margulis, 1990a), could represent the history of organisms. This was her challenge to Carl Woese's success with ribosomal gene phylogenies in reconstructing the tree of life. Larger amounts of sequence might be better, she thought, but would still fail to capture evolutionary history adequately. Molecular methods must therefore be put in their rightful place because they 'can only provide crucial independent methods to confirm or disprove evolutionary scenarios' (Margulis, 1996, p. 1075). In short, 'the techniques of molecular biology and sequence analysis by themselves are inadequate to the creation of testable evolutionary hypotheses' (Margulis, 2004, p. 173), although they may be used once such hypotheses have been developed with reference to 'organismic' and fossil data. In fact, 'definitive proof' of such an organism-based model could be provided by genomic comparison once the organism-level data had been evaluated (Margulis et al., 2006, p. 13084). This is roughly what she thought had occurred to the endosymbiosis hypothesis after she revived it in 1967.

Evolutionary scenarios should thus from Margulis's perspective be developed 'independently of molecular sequence data' (Margulis et al., 2006, p. 13080). They could only emerge from the 'meticulous study of microbes in nature, their developmental life histories, and their morphological, cell biological, and ecological relationships' (Margulis et al., 2006, p. 13084; Margulis, 2010b). Although many contemporary holobiont researchers use molecular methods and bodies of microbiome data much more liberally, in many respects their dependence on molecular insights can be aligned with organicism. How? Because holobiont molecular investigations are carried out with the principle aim of learning more about host-level processes (e.g., development, disease) rather than molecular mechanisms for their own sake.

Margulis's more extreme doubts about molecular methods did not modulate even as molecular phylogeny revealed increasingly greater amounts of detail about evolutionary history and extant biodiversity (see López-García et al., this issue). She justified her hardline stance at an even higher theoretical level. In the 1980s, she turned to autopoiesis as the 'defining principle of life' (1990a, p. 869). Autopoiesis is a concept developed to describe living systems or machines, as opposed to non-living machines (Varela et al., 1974). Its focus is self-production as a special

achievement of cells and other biological entities.⁶ Following this autopoietic view, Margulis argued that because molecules themselves were not autopoietic, they could not properly represent dynamic self-regulating systems such as cells (e.g., Margulis, 1990a). Although cells and multicellular organisms were also autopoietic systems, they had ultimately to be viewed as ‘components of the autopoietic planetary system’, in which all ‘plants, animals and microbes are connected, however circuitously, to all others spatially and by common descent’ (Margulis, 1990a, p. 869). Some components of autopoietic systems would fail to survive despite being self-producing, and neo-Darwinian natural selection could explain this. What neo-Darwinism could not explain is the persistence of the whole system or innovations within it (Margulis, 1990a, p. 870). For this autopoiesis and symbiogenesis had to be combined, she believed.

3.3 Homeostasis

‘Homeostasis’ is a common claim made in contemporary microbiome literature, but it was not discussed even implicitly when Margulis first described holobionts. However, homeostasis had already appeared very prominently in work where she – with James Lovelock – developed an account of Gaia in the late 1970s. Lovelock, an engineer with cybernetic affinities, is probably the person who introduced Margulis to the concept of self-regulatory systems. Margulis’s and Lovelock’s basic definition of homeostasis is ‘The maintenance of relatively constant conditions by active control’ (1974, p. 473). A good biological example, they suggested, is human bodily regulation of temperature. Gaia, the complex ‘totality’ of ‘atmosphere, biosphere, oceans and soils’, is best understood as ‘a feedback or cybernetic system which seeks an *optimal* physical and chemical environment for the biota’ (Margulis and Lovelock, 1974, p. 473, emphasis added).

To explain the Earth’s current geochemical optimization, Lovelock and Margulis proposed that ‘early after life began it acquired control of the planetary environment and that this homeostasis by and for the biosphere has persisted ever since’ (1974, p. 2). In other words, it was *not* ‘blind chance [continually producing] the conditions favouring the continued existence of life’ (Lovelock and Margulis, 1974, p. 2). For Margulis, this was another nail in the neo-Darwinian coffin. Often, the Gaia formulation of this homeostatic process sounds very purposeful:

‘a first task of life was to secure the environment against adverse physical and chemical change. Such security could only come from the active process of homeostasis in which unfavourable tendencies could be sensed and counter measures operated before irreversible damage had been done’ (Lovelock and Margulis, 1974, p. 8).

Margulis and Lovelock adduced their evidence for Gaia from examples such as life persisting despite global glaciation. Temperature, despite small ups and downs, ‘has followed the straight and narrow path optimal for surface life’, meaning that ‘life must actively maintain [relevant] conditions’ (Margulis and Lovelock, 1974, p. 475). It is microorganisms, they argued, that are primarily responsible for such regulatory effects, but all life participates in such maintenance. Major evolutionary perturbations, such as the Great Oxidation Event, they explained as due to the

⁶ Autopoiesis is not widely subscribed to in biological disciplines. See Scheper and Scheper (1996) for some reasons why.

greater fitness of aerobic organisms. No 'planetary engineer' was involved, however, but 'neoDarwinian mechanisms of natural selection', which produced 'larger scale modulatory mechanisms' (Margulis and Lovelock, 1974, p. 485). These mechanisms, in their view, selected organisms able to continue geochemical cycles appropriate for life even after large rises in atmospheric and eventually oceanic oxygen.

Reactions to this teleological account of biogeochemistry are predictably critical and take two main angles. The idea of Gaia as a self-regulating, self-maintaining and purposeful entity is very problematic, especially if 'lucky accidents' or byproduct explanations of stable self-perpetuating feedback are ruled out (Free and Barton, 2007). Mere stability, the main theoretical reason Lovelock and Margulis gave for homeostasis (e.g., 1974, p. 9), does not seem to require a purposeful and coordinated agent. A second line of criticism focused on the fact Gaia could not be a target of natural selection because it is not part of a population (e.g., Doolittle, 1981; see also Doolittle, 2017). Margulis herself acknowledged that the Earth, 'the largest autopoietic system [is] so far incapable of reproduction' (1990a, p. 866), and thus would not be a target of natural selection. She also later tried to clarify that Gaia is 'more homeorhetic than homeostatic, in that the internally organized system regulates around moving, rather than fixed-from-the-outside, setpoints' (Margulis, 1990a, p. 866). Homeorhesis means a system that is in a steady 'flow' as opposed to a steady state. The latter is usually the definition of homeostasis; the former has often been argued to be more appropriate for organisms. Geneticist Conrad Waddington coined the word homeorhesis, which he used for developmental processes continuing to follow a trajectory despite the occurrence of many perturbations (see Hall, 1992).

As noted above (3.2), Margulis eventually preferred the idea of autopoiesis to homeostasis for explaining how 'this planet is alive with a connected megametabolism which leads to temperature and chemical modulation systems' (1990a, p. 866). Symbiosis could also be described autopoietically, as the merger of 'heterogenous autopoietic entities' (Margulis, 1990a, p. 872). By this she simply meant that cells with genomes from different ancestries (e.g., the mitochondria in eukaryotic cells) had merged with other such cells. More broadly, Margulis marshalled autopoiesis in her ongoing crusade to refute 'mechanical, physics-centred' views of life and the world (even though the original autopoiesis accounts emphasized organisms as 'living machines'). She included in this camp of methodological mistakes neo-Darwinism, but might just as well have put cybernetic views there too despite their contribution to the original autopoiesis concept. Neo-Darwinists, according to Margulis, by seeing 'organisms as independent entities evolving by accumulation of chance mutations, *must* hate and resist an autopoietic, gaian worldview' (1990a, p. 867) Some of this resistance to conventional evolutionary views has been given new life in a broader body of recent symbiosis research.

4. Debates

The debates about holobionts today can be understood in light of the three main Margulis themes I have just outlined. These are about the nature of the entities revealed by microbiome research and whether 'holobiont' claims can be justified; the

methodological and conceptual implications of seeing microbe-host communities as unified systems (holism); and whether the states of such systems (homeostatic or non-homeostatic) is explanatorily useful.

4.1 Holobiont issues

'Holobiont' these days has become a contested term, particularly when interpreted as an evolutionarily unit of a theoretically important sort. Very briefly, these criticisms focus on whether the assemblages of entities conceived as holobionts can form cohesive units on which selection can act. Whether this is possible turns on how these entities reproduce, because heredity is central to Darwinian formulations of evolutionary units of selection. Even previously pro-holobiont concept users, the coral group mentioned earlier, have not been able to find evidence of shared heredity despite detecting some stable associations (Hester et al., 2016).⁷ Some studies do find co-adaptive dynamics between host and various microbes that result in the phylogenetic mapping of varying numbers of microbial lineages onto host lineages (Ochman et al., 2010; Moeller et al., 2016; Brucker and Bordenstein, 2012). Other investigations, however, find no phylogenetic congruence between host and microbiota, thus suggesting that appearances of such patterns is because hosts (or environments) select genes and their functions rather than lineages (e.g., Wong et al., 2013; Louca et al., 2016). Sometimes function and lineage coincide very tidily, and other times, the genes underpinning such functions may be scattered across many phylogenies (see Doolittle, this issue).

But even if there is *some* co-adaptation and co-speciation of organismal lineages, heritability of the microbiota remains low. Twin studies in humans that analyse microbiomes as quantitative traits of hosts usually find only a limited number of heritable taxa (Goodrich et al., 2014; 2016), as do breeding studies of plants and their root microbiota (Peiffer et al., 2013). Heritability may thus explain some of the congruent phylogenetic patterns. However, even when successful in finding some heritable taxa, such studies simply mean that host genetics are playing a role in determining the presence of these heritable taxa, and not that there is a common inheritance mechanism governing the reproduction of host and microbes.

Strong holobiont advocates, however, suggest different mechanisms of inheritance are at work for the whole holobiont. Those postulated include 'maternal transmission', meaning standard acquisition from the maternal environment, and more general 'coinheritance', meaning 'stable transmission from the environment' (Funkhouser and Bordenstein, 2013; Bordenstein and Theis, 2015). Some of these researchers suggest that genes from microbes will ultimately transfer into host genomes and be inherited vertically (e.g., Zilber-Rosenberg and Rosenberg, 2008; Bordenstein and Theis, 2015). This process does happen but is a rather rare evolutionary occurrence. The acquisitional nature of such 'inheritance' is why Lamarckian evolution is often loosely postulated in these claims.

Whatever the mechanism proposed, the simple fact of persistent symbiotic association is what indicates to holobiont proponents that there is an evolving unit

⁷ These authors attempt to reclaim a less conceptually loaded term of 'holobiont' for ecological analysis by distinguishing their work from 'hologenome' accounts that require or assume heredity.

consisting of a unified host and microbiota (Zilber-Rosenberg and Rosenberg 2008; Bordenstein and Theis, 2015; Gilbert et al. 2012; Bosch and McFall-Ngai, 2011). This is clearly a very different interpretation of evolutionary unit than is standard in evolutionary theory, and this is why there is controversy. Group selection in the strong holobiont view becomes community (mixed-species) selection (Gilbert et al., 2012, p. 331; Theis et al., 2016) – a far cry from the populational (single-species) groups and inheritance-related levels on which evolutionary theory is normally based. Inclusive fitness in holobionts is necessarily redefined (but never calculated) as being not about relatedness but proximity (Shropshire and Bordenstein, 2016).

Margulis herself recognized that some of her holobionts, as well as larger autopoietic systems such as Gaia, could *not* evolve in a Darwinian fashion because of not reproducing in the required way (e.g., Margulis, 1990a). However, this distinction has been lost in the general uptake of Margulis's ideas in today's holobiont-advocating research. In many other respects, however, these contemporary views mirror her message about evolution. When holobiont proponents proclaim 'for animals, as well as plants, there have never been individuals' (Gilbert et al., 2012, p. 336), they deliberately echo Margulis's dismissal of the possibility of a single eukaryotic organism (with one genome) being independent. No independent individual is alive, she argued, because no countable organism (the object of neo-Darwinian population genetics) has 'autopoietic properties' (Margulis, 1990a, p. 868). This problem lay at the crux of neo-Darwinism's supposed failure to explain eukaryotic evolution.⁸ Holobiont research has taken up this conceptual baton, but in the process has shifted the argument away from Margulis's own focus on 'conventional' or 'binary' symbioses of one host and one symbiont (e.g., Bordenstein and Theis, 2015). Much bigger and far looser aggregations of organisms have become the target. With this widening of scope has come an even stronger need to evaluate the embedded conceptualizations and theoretical claims inherited from Margulis's use of the term holobiont.

I have argued elsewhere (O'Malley, submitted) that the initial step in understanding microbiome and microbiota relationships is to assess the strength of the causal-functional interactions between specific microbes and the host, and between microbes themselves, before (perhaps) moving on to evolutionary claims. This tried-and-true approach to symbiosis research (also employed by Margulis) is required because first of all, it is not clear whether there is any physiological unity between all elements of the host-microbe system. A whole gamut of relationships is occurring between host and members of the microbiota, and between different members of the microbiota. There may well be positive, adaptive, and selected relationships between host and specific microbes (or more likely, functions that are critical to the maintenance of the relationship), but there will also be considerable variability in community composition even in short timespans, plus many non-adaptive processes and relationships. Community dynamics theorized by ecologists are able to explain the constitution and reconstitution of such communities, and even predict future states (e.g., Coyte et al., 2015). Because of the variability of causal interactions in most microbial communities and their subsequent lack of unity – a unity that is necessarily assumed in holobiont thinking – the associated Margulisian notions of holism and homeostasis become very troublesome.

⁸ Recall that Margulis meant this point only for eukaryotic and not prokaryotic evolution.

4.2 Holism issues

The holism that is detectable in microbiome research is not generally of the methodological sort Margulis advocated. Most microbiome researchers are deeply dependent on molecular techniques, and many holobiont claims are made largely on the basis of molecular data. However, the strongest holobiont positions are held by animal-microbiota researchers, where the systems seem obvious and in certain respects, indivisible. This indivisibility is not touted so extremely for physiological functions, which are often broken down mechanistically, but in relation to evolutionary unity. Microbiome research began as studies of whole communities, simply because this is how the relevant sampling works. Researchers analyse the DNA or other molecules of microbial communities in situ, be that lakes or intestines. When such samples come from, for example, the guts of animals, and those communities appear to have global effects on host physiology (despite the fact it is well-accepted there must be specific molecular mechanisms), then it can be quite easy to slip into 'holistic' system-wide claims (O'Malley, submitted; see also Huss, 2014). Once this perception of a system becomes evolutionary, implying the whole community of microbes plus the host is the unit of selection despite very limited lineage fusion, then the complaints begin (e.g., Douglas and Werren, 2016; Moran and Sloan, 2015). Holism is not confined to evolutionary claims, however, when holobionts are being conceptualized.

4.3 Homeostasis issues

Holobiont advocates plus many other microbiome researchers draw on the notion of homeostasis. The view of homeostasis Lovelock and Margulis propounded vis-à-vis Gaia is not that different from the broad homeostasis ideas floating around in the general microbiome literature (not just the holobiont-focused version of microbiota research). Microbiome homeostasis claims are even looser than the Gaia version. Mostly homeostasis is designated in a roundabout way, by focusing on 'dysbiosis'. How does this work? When there is a physiological problem in the host, it is commonly asserted that 'there is dysbiosis' in the host relationship with its microbiota (e.g., Bosch, 2012; Petersen and Round, 2014; Sonnenburg and Sonnenburg, 2014; Reid et al., 2011). Dysbiosis standardly means 'any sort of change or "imbalance" in community composition that is associated – possibly causally, but this need not be demonstrated – with illness or some other undesirable state of the host' (my interpretation of the many relaxed claims articulated in the mainstream microbiome literature).⁹ Dysbiosis is then commonly contrasted with homeostasis, which is equated with the desired – even optimal – and putatively selected health or stability state (e.g., Petersen and Round, 2014; Eberl, 2010; Neish, 2009).

How much does a Margulis-like account of homeostasis resonate in microbiota research? A surprising amount. Sometimes the word homeostasis is used so casually in contemporary research that the relationship is not very close, and merely refers to stable or health-producing states. However, in many other discussions there are invocations of self-regulatory, self-perpetuating system-level processes (e.g.,

⁹ See Olesen and Alm, 2016, for the beginnings of a critique of such statements. Vastly more conceptual analysis needs to be done on microbiome-related uses of dysbiosis, but I must leave that aside for a future paper.

Reid et al., 2011; Eberl, 2010). Mostly, this Margulisian view of homeostasis is invoked in human health microbiome research and other animal microbiome work. It is seldom used for wider microbiome research about host-free microbiota in, for example, swamps or oceans. It is not yet used a great deal for plant microbiome research, although that may change as more plant-microbe researchers jump aboard the holobiont train.

How sound is the use of homeostasis in microbiome research? Generally, it is a piece of metaphorical shorthand for a broadly 'normal' state to which the host and community return. Usually, non-homeostasis – 'dysbiosis' – is recognized on the basis of host illness. From the illness state it is inferred that the associated microbiome composition must be 'abnormal', in both physiological and evolutionary senses. The abnormal composition can be recognized by several non-equivalent measures (Debelius et al., 2016). There is almost never any direct quantitative calculation of what homeostasis or dysbiosis are (for an exception, see Casén et al. 2015): they are recognized only indirectly by variation in composition and/or host effects. Cybernetic concepts and models are rarely if ever invoked in these discussions, even when self-regulation is mentioned. Clearly, the refined formalities of such models are at a considerable remove from the current state of microbiome research, which still works with very coarse discriminations of what is going on with microbiota in hosts and elsewhere.

I noted already how Margulis turned from homeostasis to autopoiesis as a guiding concept. Does contemporary holobiont research follow Margulis's claims about autopoietic self-production? Only implicitly. Part of the reason animal microbiome researchers are so keen on the holobiont idea is probably because they see clear boundaries to their entities. Boundaries are essential to claims about autopoiesis. Microorganisms that fall within these animal boundaries are therefore simply part and parcel of them functionally and evolutionarily, as far as this thinking goes (e.g., Theis et al., 2016). However, Margulis's insistence that autopoietic systems require a 'non-mechanistic' science has not been vindicated in microbiome research more broadly, nor even in the narrower area of holobiont research. The latter area, while focused on multiorganismal units (hosts and microbial communities) takes scant notice of Margulis's methodological advice. In fact, one of the reasons holobiont researchers gain any scientific traction is because in addition to making conceptual claims, they also engage in standard parts-based mechanistic research in light of broader microbiome analyses (e.g., Bosch, 2012; Gilbert, 2014; Brucker and Bordenstein, 2012). Microbiome research in general has a long way to go in connecting parts into mechanisms and giving accounts of the activity of those mechanisms, but that is a major objective of the field. Holobiont researchers are unlikely to refuse this agenda. At least at this methodological level, therefore, Margulis has had limited influence.

5. What can we conclude about Margulis's legacy?

In general, microbiome literature uses 'holobiont' and 'homeostasis' very loosely. Likewise, any claims about 'holism' are simply directives to look at the microbes as well as the host (e.g., Gilbert et al. 2012; Bordenstein and Theis, 2015). It might be thought that such loose claims neither owe much to Margulis, or, even if they do

have their intellectual ancestry there, that they are so casual that none of the Margulis-related implications have any strength. However, this seems not to be the case when evolutionary arguments are being mounted. Assertions of the evolutionary unity of holobionts are directly influenced by Margulisian ideas, and have been acknowledged as doing so (e.g., Bordenstein and Theis, 2015; Rosenberg et al., 2009). Margulis's reluctance to understand and integrate population-genetic theory within her evolutionary work is just as apparent in this evolutionary stream of holobiont work.

But more generally, even when radical evolutionary claims are not asserted and may not even have been considered, there is still a tendency to talk about holobionts as cohesive physiological units. The same tendency can occur in wider microbiome research too. This explains in part why the field continues to focus primarily on association studies, with relationships being sought between broad community composition and general host states. General discussion of these host states brings back Margulis's ideas about homeostasis, through the supposedly opposing notion of dysbiosis. Claims about dysbiosis and homeostasis are often underpinned by evolutionary assumptions that hosts and microbiota have adaptive relationships, making them better off with one another in some sort of optimal combination. One defence of Lamarckian evolution involves the claim that microbes not working for the host will be removed from the community over evolutionary time just as non-functional genes would be removed (Rosenberg et al., 2009; however, cf. Bordenstein and Theis, 2015).

For many holobiont and other microbiome proponents, there is a strong appearance of co-adaptation and this appearance is what stimulates thoughts about the evolutionary unity of microbial symbionts and their hosts.¹⁰ But as most microbiologists and population biologists know very well, adaptation is not the only evolutionary explanation of persisting biological entities, and many microorganisms are along for the ride, short or long-term, without causing either great harm or great benefit to their host environments. Recall also that Margulis herself was focused on specific host-microbe relationships. It is only by analysing specific relationships that causal connections and evolutionary outcomes in multispecies groups can be explained (Douglas and Werren, 2016). Despite such background knowledge, many holobiont claims are based on host-microbe communities as if they were bounded in a theoretically strict way (just as in Margulis's autopoietic view). But even though Margulis focused on specific microbe-host pairs (or small groups), her broader theoretical stance has nevertheless given whole-community views a conceptual boost.

Microbiome research in general is currently maturing. Different approaches are being developed to address microbiota-host relationships. These include standard mechanistic research on how specific organismal lineages and their molecular pathways impact on pathways in the host ('bottom-up' microbiome research), in contrast to more 'top-down' approaches that include ecological analyses of host-community interactions (e.g., Coyte et al., 2015) and epidemiological analyses to determine whether causality can be inferred from association data (Cho and Blaser, 2012). These developing streams of inquiry run counter to some of the residual

¹⁰ Thanks to Alan Grafen for this point.

Margulisian legacies in holobiont research. If microbiome research as a whole is to continue to make progress, going back to evolutionary and ecological basics might be a necessary step. This does not mean Margulis has given nothing constructive to microbiome research. She certainly stimulated further thinking about microbial interactions, and put their evolutionary analysis high on the scientific agenda. But now there is increasing evidence against seeing host-microbiota as unified systems, plus many indications that holobiont ideas, when laden with Margulisian baggage, may not be the theoretical way forward. Understanding Margulis's work more fully may assist biologists and philosophers to disentangle some of her less useful ideas from contemporary research, even while continuing to recognize the impact of her pivotal JTB paper.

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