Endosymbiosis and its implications for evolutionary theory

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Historically, conceptualizations of symbiosis and endosymbiosis have been pitted against Darwinian or neo-Darwinian evolutionary theory. In more recent times, Lynn Margulis has argued vigorously along these lines. However, there are only shallow grounds for finding Darwinian concepts or population genetic theory incompatible with endosymbiosis. But is population genetics sufficiently explanatory of endosymbiosis and its role in evolution? Population genetics "follows" genes, is replication-centric, and is concerned with vertically consistent genetic lineages. It may also have explanatory limitations with regard to macroevolution. Even so, asking whether population genetics explains endosymbiosis may have the question the wrong way around. We should instead be asking how explanatory of evolution endosymbiosis is, and exactly which features of evolution it might be explaining. This paper will discuss how metabolic innovations associated with endosymbioses can drive evolution and thus provide an explanatory account of important episodes in the history of life. Metabolic explanations are both proximate and ultimate, in the same way genetic explanations are. Endosymbioses, therefore, point evolutionary biology toward an important dimension of evolutionary explanation.

endosymbiosis | evolutionary theory | macroevolution | eukaryogenesis | metabolism

Many historical accounts have viewed organelle-producing endosymbioses and symbioses in general as competing conceptually against standard evolutionary theory. Although there are several older claims to this effect, I will focus on Lynn Margulis's conjectures about how endosymbioses can be interpreted as posing problems for neo-Darwinian evolutionary theory. My aim is to assess whether endosymbiosis does in fact put pressure on evolutionary biologists and philosophers of evolution to expand beyond gene frequencies and encompass alternative explanatory frameworks.

Rather than agents of revolution bent on overthrowing evolutionary theory, it is more likely that endosymbiotic relationships offer their greatest explanatory value as model systems for macroevolution. Such systems can tell us a great deal about conflict and control dynamics in ongoing organismal interactions. They provide remarkable examples of enduring evolutionary game-changing mutualistic relationships, and call out for an account of why such relationships persist and become increasingly stable.

However, instead of focusing on "informational" properties of organisms, endosymbiotic systems draw attention to metabolism as a central organizing feature of life. A metabolic perspective focuses explanatorily on biochemical networks rather than genes, on phenotypic interactions rather than informational inheritance, on communities in addition to isolated organisms and lineages, and on major diversifications in the history of life. "Endosymbiotic" views of evolution are therefore valuable for expanding evolutionary explanations, even if they do not constitute a full-blown theoretical alternative to standard evolutionary theory.

This paper will begin with a brief discussion of how Darwinian evolutionary theory has been challenged by accounts of symbiosis and endosymbiosis, and the viability of those challenges. Lynn Margulis's claims about the deficiencies of population genetics and neo-Darwinian evolutionary theory form the contemporary focus. Although her main arguments do not withstand much analysis, macroevolutionary considerations do seem to offer an explanatory niche for endosymbiotic innovations. However, I will show that, when organelle-producing endosymbiotic relationships are scrutinized further, the explanatory focus shifts to metabolism and its evolutionary consequences. In the final part of the paper, I will revisit the implications for evolutionary theory when additional explanatory resources are gained for the modern synthesis from a metabolic interpretation of endosymbiosis.

Historical Claims About Endosymbiosis

There is a long history of researchers who have theorized about symbiosis and evolution, and many of them have aligned themselves against Darwinian evolutionary theory. Historian Jan Sapp has written a detailed history of ruminations on symbiosis as a "general principle" of evolution (1, 2). A recent contrast is provided by evolutionary microbiologist John Archibald (3), who examines historical and contemporary bodies of endosymbiosis research. Unlike Sapp's proponents, Archibald thinks there are minimal implications for evolutionary theory, despite the extraordinary importance of endosymbiosis for life on Earth. He suggests that endosymbiosis is theoretically similar to lateral gene transfer (LGT), in that it needs recognition but does not perturb the standard theoretical machinery of evolution. Working out whether endosymbiosis does have any additional explanatory purchase is the point of this paper.

An early proponent of symbiosis thinking was Constantin Mereschkowsky (1855–1921). He suggested in the early 20th century that a driving force of evolution was a biological force he called "symbiogenesis . . .the origin of organisms by the combination or by the association of two or several beings which enter into symbiosis" (2).

This "origin of organisms" (i.e., species) took several forms, and Mereschkowsky saw symbioses as major characters for delineating the kingdoms of life.

> The animal cell can thus be regarded as a simple symbiosis . . .the plant cell as a double symbiosis.. . . A third kingdom . . .the fungal kingdom [including most microbes] . . .does not represent a symbiosis. (4)

However, although this account of the origin of plants and animals was evolutionary in a general sense, and concerned with the very topic Darwin had intended to address (i.e., how species had originated), Mereschkowsky made little headway in attempts to claw theoretical ground away from Darwinian evolutionary

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theory (5). Subsequent symbiogeneticists found themselves in a similar situation, despite being more willing to concede theoretical compatibility.

Although several such efforts continued in Russian biological circles (6, 7), it was US biologist, Ivan Wallin (1883–1969), who formulated a more widely circulated criticism of Darwinian evolutionary theory. Wallin saw evolution as purposeful, and natural selection as unable to explain evolutionary creativity.

> In Natural Selection, Darwin has established [only] one of the cardinal principles [of evolution].... Natural Selection ...is the principle that controls the retention or destruction of formed species. Darwin, apparently, recognized the insufficiency of Natural Selection to produce new species and introduced other factors to fill this gap.... Natural Selection, by itself, is not sufficient to determine the direction of evolution. (8)

For this, argued Wallin, "symbionticism" was required, and his model system for how it worked was the mitochondrion.

> The establishment of intimate microsymbiotic complexes ...[called here] "Symbionticism" ...is proposed as the fundamental factor or the cardinal principle involved in the origin of species.... The basis for the postulate of the theory of Symbionticism rests upon the nature of mitochondria. (8)

Wallin understood mitochondria as symbiotic bacteria, not organelles, and because of this belief, he attempted to culture mitochondria outside the cell. He was certain he had succeeded (8), but nobody at the time or subsequently believed him. Wallin's ideas did not, however, die with his failure to convince scientists he had cultured mitochondria.

The Margulis Era of Symbiogenesis

Casting a long shadow across contemporary discussions about the relationship between endosymbiosis and standard evolutionary theory is Lynn Margulis (1938–2011). She very forcefully articulated positions on evolution that she developed in light of symbiotic relationships (e.g., ref. 9). In the process, she set out what she believed were the inadequacies of Darwinian and neo-Darwinian evolutionary theory, vis-à-vis symbiogenesis. Margulis used Mereschkowsky's term but aligned her theoretical insights with Wallin's (10). Methodologically, she identified her research, and microbiology generally, with microscopy. Although some theorists had previously argued Darwin's inadequacies lay in an overly competitive view of evolution (e.g., ref. 11), and Margulis herself did so in her earlier work, in her matured views she did not think the theoretical shortcomings of Darwinian evolutionary theory lay in its take on conflict.

> The problem is not "competition versus cooperation".... Even bankers and sports teams have to cooperate to compete. When you compete ...you still cooperate! (12)

Instead, she specified three theoretical tensions that required endosymbiotic evolutionary theory to defuse them: in population genetics, phylogeny, and the origins of novelty.

Margulis identifies the first failure of neo-Darwinian evolutionary theory as the concept of individuality that underpins the equations of population genetics.

> Lessons from symbiosis research and from molecular biology directly contradict the assumptions of mathematical evolutionary biology [because] The "individuals" handled as unities in the population equations are themselves symbiotic complexes. (13)

Margulis here is addressing what has been a philosophical conundrum for some biological theorists lately: namely, what an individual is, especially as viewed by selection (e.g., refs. 14–17). She reasons that because symbioses are fusions of at least two evolutionary lineage-forming organisms, then such entities cannot be dealt with adequately by standard methods for calculating fitness. We will see soon how population genetics does in fact manage to treat endosymbionts.

However, the problem runs deeper than that for Margulis.

> The fact that "individuals"—as the countable unities of population genetics—do not exist wreaks havoc with "cladistics," a science in which common ancestors of composite beings are supposedly rigorously determined. Failure to acknowledge the composite nature of the organisms studied invalidates entire "fields" of study. (13)

Here, the "invalidation" Margulis is asserting occurs because of violations of monophyly, which is core to phylogenetics (whether strictly cladist or not).

> In representations of standard evolutionary theory, branches on "family trees" (phylogenies) are allowed only to bifurcate. However, symbiosis analyses reveal that branches on evolutionary trees are bushy and must anastomose. (13)

Reticulation in phylogenies is a well-known problem, particularly in the prokaryote world. Molecular data now allow evolutionary microbiologists to track the evolutionary history of organisms that rarely leave traces in the fossil record (e.g., refs. 18–20). The phenomenon of LGT and the patterns it creates are not at the forefront of Margulis's complaint, however. There is no question that LGTs and endosymbioses are important phylogenetically. However, although endosymbiosis has obvious implications for the very concept of monophyly and the methodological tracking of speciation (21), this is not in the end Margulis's point: she is concerned about lateral movements of genes and cells not for phylogenetic (pattern) reasons, but for reasons to do with how evolutionary innovations are produced (process).

Margulis's ideas about evolutionary process very much emphasized the creation of novelty. She elaborated mechanistic reasons for the unease that Wallin felt with regard to whether standard evolutionary theory was in any sense "creative."

> According to present-day neo-Darwinian evolutionary theory, the only source of novelty is claimed to be by incorporation of random mutations, by recombination, gene duplication, and other DNA rearrangements. As is emphasized by those using the term symbiogenesis, symbiosis analysis contradicts these assertions by revealing "Lamarckian" cases of the inheritance of acquired genomes. (13)

This objection requires a very shallow interpretation of Lamarckianism (not much to do with the historical man's thinking) and requires that horizontal acquisitions make evolution somehow Lamarckian. However, the lateral acquisition of genes does not perturb Weismannian inheritance and Darwinian evolution because lateral transfers need to be inherited to be evolutionarily meaningful. As such, they can be treated "merely" as variation (in the way any genetic change can; see below).

Far more central to Margulis's point than giving Lamarck a role in evolutionary theory is the issue of how evolutionary novelty is generated. She is not alone in her thoughts on this, because many contemporary evolutionary theorists also concern themselves with how endosymbiosis and large-scale gene transfers contribute to evolutionary change and fit standard evolutionary models. Although not subscribing to Margulis's more radical claims, the core worry of these theorists is also whether existing theoretical accounts are sufficient to capture the biological processes that produce major evolutionary innovations (e.g., refs. 22–24). I will address this issue in detail after first dealing with neo-Darwinian rejoinders to Margulis's basic objections.

Neo-Darwinian Counterarguments

There are some fairly standard responses to the objections Margulis and her predecessors raised. Many of these counterarguments have been voiced by well-known contemporary evolutionary theorists. The first is the objection that symbiogenesis is an unwieldy elaboration of standard evolutionary theory. As

Richard Dawkins (25) famously argued in a recorded debate with Margulis on the 150th anniversary of *On the Origin of Species*,

> If you take the standard story for ordinary animals . . .what's wrong with it? You've got a distribution of animals, you've got a promontory or an island . . .so you end up with two distributions.... And then on either side of this promontory you get different selection pressures, so this one starts to evolve that way, this one starts to evolve that way, and what's wrong with that? It's highly plausible, it's economical, it's parsimonious. Why on Earth would you want to drag in symbiogenesis when it's so unparsimonious and uneconomical?

This objection looks like what philosophers would call a normative one, about the abstracted knowledge-making virtues of the explanation (not the substance). Unfortunately, such objections leave themselves wide open to Margulis's reply, which was supposedly emphasizing substance: "Because it's there" (26).

Margulis's rejoinder is often interpreted to mean that facts are facts, and ruling them out on the rarefied grounds of parsimony will not get rid of them. However, Dawkins is perfectly willing to accept that symbiogenesis has occasional evolutionarily important outcomes: he says so during the same debate (25). His objection is that symbiogenetic events are not causal regularities that can be modeled theoretically (asteroid impacts would probably fit this category too). Margulis does seem to be implying that symbiogenesis is the right sort of causal regularity (26). She is suggesting that standard evolutionary theory is seriously challenged because it has made substantial errors about what is going on in a regular mechanistic way in evolution. This is what she means when she attacks the idea of mutation as sufficient for understanding evolutionary novelty.

Although Dawkins suggests endosymbiosis has no role to play theoretically (which does not mean empirically), a more strategic move is to say it is already included and in a perfectly adequate way. This is what John Maynard Smith (1920–2004) proposed: "The relevance of symbiosis is that it affords a mechanism whereby genetic material from very distantly related organisms can be brought together in a single descendant" (27).

We will come back to the implications of "mechanism" soon, but Maynard Smith's point here is that endosymbiosis can be treated as a large mutational change (or a rapid series of smaller mutations). Multiple mutations occur, for whatever reason (endosymbiosis or hypermutation of some sort), and then the standard machinery of population genetics can be applied to the populations possessing such mutations.

There is a large body of population genetic research on endosymbionts and organelles that works in this way, by analyzing gene frequencies that occur after the original "mutational" event. These populational studies focus on the genetics of mitochondria, plastids, and maternally inherited endosymbionts such as *Buchnera* and *Wolbachia*. Many useful questions can be asked about the forces that shape the evolution of endosymbiont genomes: whether selection, drift, or mutational pressure have reconfigured the populations of these endosymbionts; why there are different patterns of genome reduction in different endosymbionts; whether organelle genomes evolve adaptively; and whether deviations from neutral evolution have consequences for phylogenetic and biogeographical analyses based on organellar DNA (e.g., refs. 28–35). It is studies such as these that allowed Maynard Smith to conceive of endosymbiont population genetics as standardly explained evolution.

However, this research by and large treats endosymbionts, organelles, and hosts separately, as distinct genetic individuals (which is Margulis's first complaint above). In some circumstances, however, host and endosymbiont population genetics may be discordant, and this is where some very interesting explanatory attention focuses. Different patterns of genetic variation in nuclear, mitochondrial, and plastid genomes mean that all three lineages of genomes need population-genetic analysis to describe and explain the composite population structure (e.g., refs. 36–39). This works against Margulis's assertion these "individuals" cannot be combined, although combination is not a simple matter of data aggregation (40). Despite the problems of merging what could be distinct evolutionary histories, much progress has been made in teasing out different causal processes at multiple levels of organization, and their impacts on organelles, endosymbionts, and host genomes, separately and together (41). However, there are still grounds for Margulis's third objection: how do these theorists see the actual incoming genetic event of endosymbiosis, and can they explain it?

Once again, the Maynard Smith strategy of "incorporation" removes some of the sting of this problem. He would simply describe endosymbiotic contributions as "macromutations." These are mutations that bring about large-effect changes in genetic variation but are fully compatible with "micromutation" (42–44). Earlier advocacy of macromutation by "heretics" such as Richard Goldschmidt is not what is meant here, although most macromutation discussions acknowledge Goldschmidt's earlier use of the term (see ref. 43 for a categorization of different meanings of macromutation). The modern synthesis initially allowed only mutational changes with small effects (44), but Maynard Smith (42) argues this need not be the neo-Darwinian position. As he elaborates (42),

> It is convenient to use the term *macromutation* for any genetic change leading to a striking change in phenotype, even if the change is a point mutation. Such macromutations are likely to be ill-adapted until compensating changes have occurred at other loci.

However—and this is important for the evolutionary position Margulis and earlier endosymbiosis proponents held—there may be an appearance of discontinuity, which is attributed to rapid effects of micromutations. The debate thus turns on whether macroevolution requires the same explanation as microevolution. Endosymbiosis theorists keep interesting company in their belief that the former cannot be reduced explanatorily to the latter.

Microevolution "Versus" Macroevolution

The history of the modern synthesis (and earlier) has numerous episodes in which microevolution is discussed in relation to larger-scale macroevolutionary patterns. Conceptually,

> "Microevolution" is typically understood as evolution "below the species level" including . . .mutation, recombination, gene flow, drift, natural selection, local interaction among species (such as parasitism, predation and competition), and mechanisms of speciation. By contrast, the macroevolutionary domain includes phenomena at larger temporal, geographical and taxonomic scales, such as . . .mass extinctions, long-term diversification patterns, geographical and temporal patterns in the origination of major evolutionary novelties. (45)

George Gaylord Simpson (1902–1984), a key theorist of the modern synthesis, felt that Theodosius Dobzhansky's founding announcement—that macroevolution is microevolution over longer timescales—needed elaboration. Simpson coined new terminology of "mega-evolution" and "quantum evolution." He suggested that microevolution is about changes in continuous populations, whereas macroevolution is concerned with the formation of "continuous groups" (46). However, beyond even macroevolution, Simpson argued that megaevolution should be the focus of paleontology, because it was concerned with discontinuities that were not only lengthy (and rare), but also less likely to be filled in by future fossil discoveries (46).

Although Simpson thought megaevolution was relevant to the origin of major clades at high levels of the taxonomic hierarchy, nevertheless "the materials and factors inducing and directing it [are] the same at all levels . . .and differ in mega-evolution only in intensity and combination" (46). Quantum evolution was for Simpson part of a more precise account of the three modes of

evolution underlying different evolutionary patterns. Unlike the first two modes, of speciation and phyletic evolution, quantum evolution explains the origins of the most inclusive clades, such as families, orders, and classes (46). Organisms fall out of equilibrium with their environments, go through maladaptive phases, and then (if extinction does not occur) shift in a "quantum" way to new adaptive equilibria. However, despite the quantum leap, Simpson thought the causes were the same, and the events not rapid enough for "instantaneous" change (46). In later work (after collegial censure), he further downplayed these ideas about quantum evolution, so that macroevolution was theorized much more straightforwardly as the ongoing accumulation of microevolutionary change (47).

Simpson's strategy has been elaborated on by contemporary evolutionary biologists conceptualizing macroevolution. For example, evolutionary microbiologist Thomas Cavalier-Smith takes up the idea of quantum evolution to describe the uneven pace of major innovations in evolution, such as eukaryogenesis: "Major cellular innovations exhibit a pattern of quantum evolution followed by very rapid radiation and then substantial stasis, as described by Simpson" (48).

Change may not be uniform, but even abrupt major transformations occur against a backdrop of gradual mutational change. These breaks in pattern can be explained mechanistically by standard micromutational evolutionary theory (48). However, Cavalier-Smith also notes that rare membrane innovations are causal contributors to macroevolutionary events, and that most of these innovations are the product of symbiogenesis (49). From his perspective, however, eukaryogenesis and its intracellular innovations are initiated by "internal" gene-based transformations rather than "external" symbiogenetic acquisitions. We will see below how not all accounts of eukaryogenesis attribute it to the mutational forces that Cavalier-Smith sees as the main driver of this evolutionary transition, and how for some theorists another mechanistic process has to be invoked that goes beyond membranes in and of themselves.

However, for the majority of population geneticists and general evolutionary theorists, "There is no evidence suggesting the need for qualitatively new mechanisms to account for macroevolutionary patterns" (50). In other words, gene flow, mutations, recombination, and natural selection (including sexual selection), plus time, are sufficient to explain each and every evolutionary pattern. From a strong neo-Darwinian angle, as Maynard Smith argued, endosymbioses are treatable as standard mutations, and macromutations are perfectly compatible with micromutations. Both the process of variation and the frequencies of variants are accounted for by this strategy.

Explanatory Sufficiency and Reducibility

Not everyone agrees with such assessments, and there are skeptical responses that challenge the explanatory sufficiency of population genetics. For example,

> microevolutionary studies generally show that micromutation, genetic drift, and selection are at least *capable* of contributing to macroevolutionary trends. Whether or not microevolution is the major determinant of evolutionary diversification will likely remain a matter of debate [because of the difficulties of observing macroevolutionary events]. (51)

"Contributing to an explanation" and "being sufficient to explain" are not the same, obviously, and giving other processes an explanatory role is what the debate is about. A particular sticking point is the pace at which evolution happens, and whether mutational contributions are fast enough to explain seemingly accelerated periods of evolutionary history. For instance, LGT is sometimes proposed as the mechanism of "big-bang" phases of evolution (52), and endosymbiosis could be viewed as a very large transfer of genes in such a framework. Some theorists counsel going back to Wallin for theoretical ideas about assessing rates of evolutionary change in relation to symbiotic events.

> [We need] to formulate a coherent theory of speciation that includes both genes and symbionts . . .to assess whether symbionts accelerate the evolution of reproductive barriers [compared to] other causes of reproductive barriers. (24)

There are some much more radically skeptical responses than these, however, and symbiogeneticists like Margulis are not alone in doubting the sufficiency of genetic analyses to explain the history of life. Paleontologists historically have felt strong affiliations to the position that microevolutionary processes are unable to explain macroevolutionary patterns (e.g., refs. 53–55). Stephen Jay Gould and Niles Eldredge advocated punctuated equilibrium theory to explain what they saw as otherwise inexplicable macroevolutionary patterns (56). Gould in particular thought that Simpson had betrayed his discipline by arguing for the continuity of microevolution and macroevolution (47). Gould held both that these were qualitatively different sorts of events and that they would have a qualitatively different explanation from micromutational evolutionary theory (53).

Todd Grantham (45), a philosopher of paleontology, has outlined the three positions into which the microevolutionary versus macroevolutionary debate can fall: (*i*) macroevolution can be reduced explanatorily to microevolution (with no remainder); (*ii*) macroevolution is "in principle" explanatorily reducible to microevolutionary processes, but theorists need to focus on the macro side to put together sufficiently complex combinations of microprocesses; and (*iii*) macroevolution cannot be reduced explanatorily to microevolution.

It should be clear by now that it is in this third position that strong symbiogenesis proponents place themselves, but for different reasons from the paleontologists (not discontinuity in the fossil record, although that can come into the argument secondarily). One of the key reasons already identified is the process or mechanism by which novelty is produced, and this has a corollary: the speed at which major evolutionary changes occur. Margulis would be willing to agree that micromutations produce gradual change, but would think that genuinely creative macromutations could only be produced by processes such as symbiogenesis. These are much more likely to happen quickly, runs this argument, and thereby bring about saltational evolutionary events (57).

Margulis thus aligns herself with the irreducible option: "Symbiogenesis, interspecific fusions (hybridogenesis, gene transfers . . . and other forms of acquisition of "foreign genomes" or epigenesis) are more important than the slow gradual accumulation of mutations or sexual mergers" (12).

This statement looks like it leaves room for compatibility with "lower-level" standard evolutionary theory, but does not agree with the reducibility of macro- to microevolution. Crucially, Margulis sees a major evidential gap in what microevolutionary theory has achieved.

> The gradual passage from an ancestral to a descendant species by the accumulation of random mutations has not been demonstrated in the field, nor in the laboratory, nor in the fossil record. Instead, symbiogenesis [fills this gap]. (58)

Margulis's version of the "irreducible" option is presented in a way Gould would have been happy with, although it is worded very dangerously as far as intelligent-design uptake goes. She also argues that symbiogenesis explains punctuated equilibrium (58), bringing symbiogeneticists even closer in theoretical alignment with paleontologists.

For both paleobiologists and endosymbiosis theorists, the obvious tensions that play key roles in separating microevolutionary from macroevolutionary explanations are allegedly distinct tempos and modes of evolution: gradual versus rapid evolutionary changes;

random variation and its destruction versus creative innovation. One of the clearest examples of how these tensions play out is in explanations for the origin of eukaryotes. This evolutionary transition is also a major breeding ground—as we have already seen—for endosymbiotic accounts of evolution.

The Origin of Eukaryotes

There are very roughly two main hypotheses for the evolution of eukaryotes: one sees the process as mutation-driven, with lateral acquisitions of genes and organisms also involved but in a causally secondary way (e.g., refs. 59 and 60); the other sees eukaryogenesis as driven causally by the acquisition of the mitochondrion (e.g., refs. 61 and 62). Mutations are common events, and usually involve small changes because large ones are likely to be deleterious (42). The acquisition of the mitochondrion, however, is often portrayed as a one-off event that instigated a rapid transformation with major evolutionary outcomes (e.g., refs. 63 and 64). Currently, the evidential verdict is still in the balance between mutation- and endosymbiosis-driven explanations of eukaryogenesis, in part because of shifting ideas about the natures of the host, the symbiont, their precursor structures, and the subsequent sequence of cellular transformations (65–68). Although the explanatory tension between gradualism and rapid evolutionary change is important to these debates, and to the theoretical importance of endosymbiosis, it is not unreasonable to assume that any evolutionary event will fall along a spectrum of minor to major change, and that different combinations of explanatory factors will have to be invoked to account for such changes and their evolutionary impact.

A better way of asking the question about eukaryogenesis is not whether one explanation can be boiled down to the other (i.e., reducibility), but whether in explaining the emergence of major biological groups with new capacities, population genetics can carry the whole explanatory load. Another explanatory strategy might be required: one that encompasses what a population genetics explanation does not, cannot, and was never intended to cover. This strategy fits the second position outlined by Grantham above (45), in which microevolutionary explanations can be elaborated and expanded by additional explanations of macroevolutionary phenomena. Although evolutionary events can always be explained in terms of genes, doing so can often account only in the most minimal way for evolutionary transitions and other major evolutionary turning points. Only certain explanatory goals are achieved by a gene-based strategy. In other words, there are additional causal cascades—some of them causally impacting on genes—that need consideration before conceding that eukaryogenesis has been explained. A full-blown explanation of eukaryogenesis would need to capture the major causal processes driving relevant shifts in gene frequencies. Genetic analyses will of course strengthen inferences about the causal forces involved in processes such as eukaryogenesis, but may not always be first in the explanatory queue.

However, even if we agree a supplementary explanatory strategy might be useful, will symbiogenesis itself do the necessary explanatory work? In other words, does symbiogenesis capture the causal difference makers missing from a strictly genetic account? As Zimorski et al. (67) put it, "the real strength of [evolutionary endosymbiont] theory is that it accounts for the physiological and biochemical similarity of organelles to prokaryote cells." Explaining similarity is not, however, the same as causally explaining eukaryogenesis. Given that concession, symbiogenesis might be more like a condition for the core causal processes driving such evolutionary transformations, rather than the central difference-making mechanism. What then would the relevant mechanism be? Returning to the eukaryogenesis example, neither population genetics nor symbiogenesis seem better off at explaining how early eukaryotes overcame the conflict between lower-level entities and the new higher-level one (69). These frameworks also cannot explain why there is no pressure now on prokaryotes to gain eukaryotic features (i.e., large genomes, intracellular complexity), even when there are prokaryotes in small populations with concomitantly larger tolerances of mutations (70). However, an aspect of endosymbiosis—membrane-based metabolism—does seem to be able to achieve these explanatory goals.

Metabolic Evolutionary Explanation

If there is a missing link, at least in macroevolutionary accounts such as those concerned with major turning points in evolution, it is more plausibly metabolic than "symbiogenetic": about biologically structured energy conversion. An important explanatory step that needs to be made for eukaryogenesis and probably other important innovations in the history of evolution is to place mechanistic metabolic explanations alongside statistical genetic ones. A recent explanatory effort argues along these lines, by focusing on the energetic leap made by cells that acquired the mitochondrion with its energy-enhancing membranes and retention of genes to control energy generation.

> The transition to [eukaryotes] was a unique event that hinged on a bioenergetic jump afforded by spatially combinatorial relations between two cells and two genomes (endosymbiosis), rather than natural selection acting on mutations accumulated gradually among physically isolated prokaryotic individuals. (70)

Now of course gene-based methods can track that event (i.e., allow causal inferences to be made retrospectively). In addition, some key differences between prokaryotes and eukaryotes can be explained by a general statistical evolutionary account (71) of the relationship between population size and genome architecture (72, 73). This account does not, however, explain or attempt to explain eukaryogenesis.

A metabolic perspective on evolution such as the one above (70) does attempt to provide a causal mechanistic account of eukaryogenesis, by specifying the causal forces precipitating it. In this explanation, endosymbiosis can be understood primarily as a condition for the evolution of novel membrane-dependent metabolic capabilities (this is what "afford" might mean in the quote above). Whether we believe mitochondrion-first or mitochondrion-last hypotheses of eukaryogenesis, this causal contribution is a sine qua non of being a eukaryote (3, 74). By giving metabolic factors explanatory weight alongside genes, it becomes possible to explain capacities for baroque genomes, greater cell size, and increased intracellular complexity (70), as well as conflict mediation between different levels of selection (69).

Suggesting that metabolism might causally impact on genes and their frequencies does not immediately seem to fit the standard causal flow of evolutionary or even nonevolutionary explanations. However, there are numerous circumstances in which metabolic processes causally control genetic ones (75–77), despite the fact that doing so requires a reversal of the normal explanatory order. The redox control of transcription in chloroplast-bearing cells is a clear illustration of how such a "reverse" explanation can work (77). However, this is more than a proximate mechanism because it explains evolutionarily why plastids and mitochondria maintain genomes (78), and also how the conflict dynamics in the early days of mitochondrial and plastid assimilation might have been mediated metabolically (69, 79).

Cavalier-Smith (49) agrees symbiogenesis can be important, but that it happened only a few times and is therefore not enough of a regularity to fret about theoretically (similar to Dawkins's point above). However, there are many major turning points in the history of life that also benefit from metabolic explanations. Not all of them are endosymbiotic. For example, the Permian extinction, which was an event of major evolutionary importance for larger life forms, can be explained by metabolic capacities. Specifically, an upheaval of the existing carbon cycle can be attributed to a metabolic innovation in methane-producing prokaryotes via LGT

(80). Although the gene transfer is indeed a causal explanation in its own right (one that can then be taken up by a statistical population genetic explanation), the full causal explanation relies on the mechanism of a metabolic pathway. This mechanism changed the phenotype of the recipient organisms, whose subsequent metabolic activities then had a massive impact on the rest of the world's geochemistry and biota. Genes are good explanations of certain aspects of events but cannot in this situation explain why such a devastating extinction happened. Metabolic interactions are what made the causal difference in this and many other biogeochemical revolutions.

Another major turning point in the history of life is the divergence of Bacteria and Archaea, perhaps best understood as the primordial bifurcation of extant life. Recent analysis suggests that the core differences between archaeal and bacterial membranes can best be explained bioenergetically—as consequences of chance divergences in separate populations, when initially similar protocells gained different ion-pumping membranes (81). Those membrane divergences can of course be explained genetically too, but that will be a very limited evolutionary explanation. In fact, this particular piece of research (81) starts by assuming an ultimate causal explanation will necessarily go beyond gene frequencies. Supplementing the genetic explanation with a metabolic one is thus required to account for the mechanism that caused the most basal diversification of life.

The origin of life is a paradigm case of how genetic and metabolic explanations both need addressing. Even the strongest metabolism-first or information-first origin theorist would acknowledge that a complete explanation of early life requires a combination of perspectives. Some of these combinations may simply define metabolism as "prebiotic" or before cellular life (e.g., ref. 82), whereas others may suggest metabolic factors causally underpin replicating material (e.g., ref. 83). However, no account will dismiss the importance of the unification of replication and metabolism for a full evolutionary explanation of emerging cellular life (84). In addition, in whichever era of evolution metabolism plays an explanatory role, it is not merely a proximate explanation that enables the ultimate evolutionary genetic explanations.

What evolutionary considerations of eukaryogenesis and other metabolic mechanisms suggest is an additional, causally extended explanatory strategy. Genetic explanations tell only the minimal story of what happened and why. The extended "why" requires the inclusion of phenotypic mechanisms that causally affect genes and their distributions. The modern synthesis is not by any means opposed to the inclusion of metabolic mechanisms, but these would be deemed proximate, rather than ultimate. In other words, Ernst Mayr's (85) distinction between "proximate" and "ultimate" biological causes would categorize metabolic factors as merely proximal: conditions that made evolutionary changes possible, but that do not causally or statistically explain why such events had the evolutionary outcomes they did. Evolutionary theorists see their task as the provision of ultimate explanations of microevolutionary and macroevolutionary events via models of the evolutionary forces driving them. Metabolic and any other proximate factors from this perspective are mere contributors to the relevant explanatory background; as phenotypes, metabolisms are judged not to have the relevant "genotypic" properties of heritability, and thus to be unable to function as ultimate causal mechanisms.

However, as Lynch et al. (86) observe, "a full mechanistic understanding of evolutionary processes will never be achieved without an elucidation of how cellular features become established and modified." Metabolism in this light is a general mechanism that contributes to ultimate explanations. The metabolic innovations brought about by organelle-producing endosymbioses utterly changed existing selective regimes (87). Mitochondrial and plastid metabolic contributions made possible new modes of life that spread across the tree of life and created major new branches. Metabolism in numerous other evolutionary events both enabled and constrained genetic novelty (88), which can then be addressed by standard statistical explanations.

Different Explanatory Aims

Although many major events in the history of life have ultimate metabolic explanations as well as genetic ones (e.g., life's origins, the Bacteria–Archaea split, eukaryogenesis, plastid acquisition, multicellularity), it is probably not the case that every significant evolutionary turning point needs to be explained metabolically (perhaps protocells evolving a division of labor between nucleic and amino acids is one such instance). Other suggestions for ultimate causal explanatory factors are cellular structures that allow different trophic modes such as predatory, parasitic, osmotrophic, and mixotrophic feeding (89). Worden et al. (89) argue, as they explain primal eukaryotic innovations, "Metabolism may be diverse, but environmental interactions are strongly guided by cellular structures and the behaviors they underpin." They are suggesting it is not possible to restrict these feeding behaviors to genetic or metabolic explanations and that cellular structures comprise another explanatory strategy. Rather than suggesting that feeding strategies might be a type of metabolic explanation, this additional angle is probably best understood as adding weight to the broad idea that large-scale evolutionary events need additional explanatory strategies. As one proponent of metabolic evolutionary explanations notes (90), eventually "lifestyle became much more important than energy-per-gene as the major determinant of genome size [and intracellular complexity] in eukaryotes." However, for at least some theorists, breaching prokaryotic constraints initially required energy increase via membrane-based metabolism, and that is what made the difference in the evolutionary emergence of eukaryotes.

The aim of my discussion is to advance a general argument for metabolism-based evolutionary mechanisms, rather than to give a series of detailed metabolic explanations. At this general explanatory level, it is possible to compare what a population-genetic or other gene-based explanation achieves, and what a metabolic explanation achieves. Deciding that one and only one explanation applies may work in some evolutionary instances, but not in the cases of endosymbiosis that produced organelles, nor even of other endosymbioses and symbioses that have persisted for millions of years. Seeking explanatory parity for metabolism requires the recognition that

> There is more to metabolism than can be captured in the selfishness of genes or compartments: . . .the metabolic character of life . . .may predate [genetic] individuality. We [may] want to think of metabolism as more than a follower of genes—and in many respects, we will want to think of it as leader. (91)

The endosymbiotic side of Table 1 is labeled that way simply to address the traditional debate (which is the starting point of

Table 1. The explanatory emphases of standard and endosymbiotic evolutionary theories

Standard	"Endosymbiotic"
Gene-centric	Metabolism-centric
Informational	Biochemical; energetic
Populations	Communities
Cumulative evolutionary change, both microevolutionary and macroevolutionary	Major transitions and other seemingly abrupt macroevolutionary events
Predictable processes of mutational change (small and large)	Generalizable combinations of causes (mechanisms)
Statistical explanation (population genetics)	Causal mechanistic explanation

this paper), but the various factors that comprise it are—I suggest—what Margulis and other endosymbiosis theorists are getting at explanatorily. Gene-centric explanations may work for many aspects of macroevolutionary events. However, at several world-changing nodes in the history of life, metabolic mechanisms have to be factored into explanations of why there are eukaryote cells and plastid-bearing eukaryotes, why endosymbionts are evolutionarily important, and why organelles gave rise to novel modes of life and new evolutionary opportunities. Although Margulis's account does not foreground the metabolic nature of endosymbiotic contributions to evolutionary theory, her view is a useful stepping-stone to a more multilevel explanatory account of major and ongoing events in evolutionary history.

The final question to ask is about the theoretical importance of a metabolic explanatory strategy. Do the considerations above add up to a new theoretical perspective, such that the claims of more radical symbiogenesis proponents might be vindicated? Even though the explanatory complement is not symbiogenesis per se, but metabolism, some metabolism-oriented commentators have suggested that a bigger theoretical revision is in the offing. For example, "[there may be] a grand unified theory for at least the core of metabolism, which links ecology, biochemistry, and the Darwinian world into a whole larger than any one of them" (91).

Tempting as it may be to try and piece together a grander evolutionary account (and this is the temptation to which Margulis unwisely succumbed), the state of play is currently one in which it is more reasonable to suggest a metabolically informed supplement to microevolutionary explanation. This is not, however, merely an optional extra. Metabolism constrains what genetic information can achieve; it also provides the evolutionary potential for genes. However, even if there is no grand fully fledged theory on offer now or even in the future, evolutionary theorists might at least see reasons to balance up their gene-centric explanations of evolution with metabolic ones.

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