

Reproduction Expanded: Multigenerational and Multilineal Units of Evolution

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Reproduction is central to biology and evolution. Standard concepts of reproduction are drawn from animals. Nonstandard examples of reproduction can be found in unicellular eukaryotes that distribute their reproductive strategies across multiple generations, and in mutualistic systems that combine different modes of reproduction across multiple lineages. Examining multigenerational and multilineal reproducers and how they align fitness has implications for conceptualizing units of evolution.

1. Introduction. Reproduction is a phenomenon central to life on Earth. In many respects it can be straightforwardly understood as the multiplication of organismal units, such that there is clear continuity between parent and offspring. A basic account like this slots neatly into neo-Darwinian evolutionary theory, in which reproductive success is both the *modus operandum* and measure of evolution by natural selection. In what follows, I will suggest that this standard view does not incorporate everything that could and possibly should be said about reproduction as a biological phenomenon, nor is it the full story of the relationship between reproduction and evolution. I will make these points with two sorts of examples: eukaryotic microbes (commonly called protists) and communal entities that comprise microbes and multicellular eukaryotes. Both these entities stretch the concept of reproduction (and life cycle) in ways that have ramifications for how evolutionary units are conceptualized.

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Using a heuristic that seems to be quite useful for philosophy of biology—namely, that starting with microbes is a better strategy than beginning with the rarer organisms more like ourselves (O'Malley 2014)—I will examine two modes of reproduction that are strangely and importantly different from that of our own. The first one I call 'multigenerational reproduction', in which protists deploy a variety of reproductive strategies across several generations. These strategies have come to be combined in one generation in organisms such as animals, and I will offer some ideas about how this has happened. The second mode of reproduction is 'multilineal reproduction', in which microbes and multicellular entities work as functionally cohesive units over generational and evolutionary time. I will draw conclusions from both modes of reproduction about evolutionary continuants that align fitness and maintain functional consistency over time. In short, this analysis of reproduction points beyond Darwinian individuals as the relevant evolutionary unit.

2. Why Reproduction? Without reproduction, there would be a very different kind of biology on the planet. Although it is possible to identify non-reproducing but persisting biological entities (e.g., Bouchard 2011), by and large this is not how we see biology working. Multiplication and continuity remain central to the world we know. And crucially, to understand evolution conceptually and empirically, we need to understand reproduction (Godfrey-Smith 2013). Above and beyond understanding what organisms are, evolution requires the identification of units of selection, or Darwinian individuals (Godfrey-Smith 2009). In fact, 'Darwinian individuals are essentially *reproducing* things' (Booth 2014, 664), because fitness-bearers need to reproduce to demonstrate their fitness.

I am not questioning this line of reasoning. My doubts are about whether a standard concept of reproduction, restricted to the multiplication of organisms via strict parent–offspring relationships, is sufficient for the purpose of identifying all units of evolutionary relevance. One concern might be that evolutionary theory in the past has exhibited a strong tendency to work downward from animals and exclude biological life-forms that do not fit animal-derived concepts (O'Malley 2014). What sort of concept of reproduction do we need if it is to apply across the tree of life? The other concern is whether reproduction is central only or primarily to Darwinian individuality, or whether it might also have relevance to other units participating in evolution. Ultimately, I will suggest that we need to think beyond Darwinian individuals to understand reproduction fully.

Several criteria have been used to delineate reproducing individuals in order to identify their theoretical relevance to evolutionary theory. Bottlenecks are very important to this endeavor. In Richard Dawkins's influential dictum, "The essential, defining feature of an individual organism *is* that it is a unit

that begins and ends with a single-celled bottleneck” (1976/2006, 264). Bottlenecks allow organismal lineages to make a fresh start by expelling genetic parasites or mutations in somatic cells. Bottlenecks also allow small changes to have major effects on offspring, such that reproductive success is affected.

Having a bottleneck usually entails the existence of germ lines, which means that only some parts of the collective multicellular entity reproduce. These specialized reproductive cells are responsible for carrying the heritable properties of the organism into future generations. Both bottlenecks and germ lines are central to what we might call the standard view of reproduction. It seems to work for animals such as ourselves. That is no surprise, since these criteria are derived from considerations of fairly simple life cycles in animals. Peter Godfrey-Smith (2013) has emphasized both these features in his reflections on reproduction and Darwinian individuality. The question is whether these criteria work for complex life cycles in unicellular organisms and multicellular collectives, and whether it matters if they do not. Put another way, the main thing about bottlenecks and germ lines is that they function to realign fitness, and numerous evolutionary individuals have realized such alignment without these features (Wilson and Sober 1989). Fitness alignment at its most general is a process whereby reproducing entities minimize conflict and maximize fitness. Are animal-like bottlenecks and germ lines the only way by which fitness alignment can be achieved? In the examples that follow I will suggest not, from both a multigenerational and a multilineal perspective.

3. Multigenerational Reproduction. All reproduction leads to multiple generations, as organisms produce at least one more of themselves. My use of ‘multigenerational reproduction’ means something else, however. It is a process whereby different mechanisms of fitness alignment are deployed in different generations of an organismal lineage. Many instances of such deployment can be found in protists, in which sex and reproduction are often decoupled. A nice example of this can be seen in the green alga, *Volvox carteri*, which has featured in several philosophical discussions of individuality (e.g., Godfrey-Smith 2009; Herron et al. 2013). *V. carteri* are beautiful multicellular organisms that reproduce asexually. A large haploid spheroid gives birth to small haploid spheroids, all of which mature into the larger forms.¹ But every now and then, ordinarily when their aquatic habitats dry up, *V. carteri* instead produce sexual gametes that fuse and form a resilient

1. There may also be no bottleneck during asexual reproduction, if the multicellular spheroid is the reproducer. However, some commentators consider the internal elements of the spheroid to be the reproducers. Because early on these are single cells (within a multicellular entity), each single cell is deemed to be a bottleneck.

diploid zygospore (Kirk 2001). The zygospores remain dormant until conditions improve, at which time asexual reproduction begins again.

The sexual phase of the life cycle is nonreproductive because there is no multiplication (the opposite, because two sexual cells produce only one zygospore). It is not part of a single 'life cycle' either, because sex happens only between what might be many asexual multiplicative generations. In *Volvox* and other protists, processes that define reproduction for animals (e.g., sex) are commonly facultative (nonobligatory) and biologically separated from multiplication. 'Life cycle' might therefore require conceptual stretching to accommodate multigenerational sequences of events.

An example that magnifies such differences can be found in ciliates, which include well-known laboratory models such as *Paramecium* and *Tetrahymena*. All ciliates have a micronucleus, which provides the germ line, and a macronucleus, which is responsible for all the growth and asexual reproductive functions of the organism. A single species of ciliate can have from two to 100 mating types or sexes, and at least three modes of inheritance (Phadke and Zufall 2009). Sexual recombination occurs between asexual generations and does not lead to reproduction (of the cell). However, sex does involve the reproduction of the nucleus via fusion, which in most multicellular eukaryotes co-occurs with sex-cell fusion. The sexual process happens only occasionally in ciliates and other protists, between hundreds or even thousands of asexual generations (Ene and Bennett 2014).

One way to deal with ciliates is to conceptualize sexual nonreproduction as merely a blip in a lineage's normal reproductive history. One reason against this is that occasional sexual episodes are a characteristic occurrence in all ciliates. A second reason is that sex appears to be a mechanism of aligning fitness across generations of cells. In the sexual process, the genome is reconstructed and selfish genetic elements evicted (Bracht et al. 2013). Even though ciliate sex occurs outside reproduction (in a unigenerational sense), it serves a very similar multigenerational function to traditional bottlenecks. However, this mechanism is a more complex strategy than the bottlenecking seen in animals. Most notably, it is multigenerational, which implies that the reproducing unit is also multigenerational.

An extreme example of reproductive complexity is *Physarum polycephalum*, the 'acellular' slime mould.² *Physarum* are fascinating organisms that have been used in maze-navigation and anticipation experiments, where they perform remarkably well. They also combine an impressive array of reproductive developmental strategies into a multigenerational life cycle. These strategies include a diploid coenocyte (which sometimes has a dormant stage),

2. They are 'acellular' because as they 'grow', the nuclei divide and thus multiply, but the dividing cells fuse. These expanded cells are called 'coenocytes' (and in this case 'plasmodia'). Coenocytes begin as cells that undergo mitosis but not cytokinesis.

followed by sporulation, spore release, and the ‘birth’ of amoebae from spores. These amoebae are haploid gametes that can form cysts and sometimes develop flagella. The gametes mostly mate, which creates two uninucleate progeny that develop into multinucleate coenocytes all over again. In certain conditions, fragmented plasmodia and spherules can develop from coenocytes. It is hard to imagine a more complex, developmentally diverse life cycle than this. Each subcycle of this multigenerational life cycle exhibits changes in gene expression, cellular organization, and behavior (Bailey 1997). It would be impossible to understand what *Physarum* is as an organism and an evolving entity without considering all these stages.

One of the conclusions such extended life cycles point to is that the functions of sex and development might be better understood across multiple generations than they are in single generations (Ene and Bennett 2014; Grieseimer 2014). The varieties of modes of sexuality (mating types) across protists suggest that reaching a two-sex system in a species is a reduction of something more diverse in earlier evolution. From a multigenerational perspective, sex realigns fitness by purging and restructuring genomes. Sex itself appears to be a flexible developmental strategy that involves development over generations of the population, rather than the ‘individual organism’ life cycle.

Consequently, when we add the complexities of protist sex and reproduction to a general concept of reproduction, we also have to rethink development in a more generalizable way. Development is not an ‘infant-to-adult’ progression but something more diverse (Minelli 2011). It produces different reproductive capacities, different morphologies, different genomic structures, and different individual evolutionary trajectories. Only by understanding multigenerational individuals do we gain a sense of what is developing in these protist examples. In addition, in some protist groups, bottlenecks of a conventional sort (in which multicells are reduced to single cells) are not always conjoined with germ–soma distinctions (Herron et al. 2013).³ Thus, the standard view of one entailing the other may be true only in some types of organism.

What are the implications of recognizing these reproductive diversities? Perhaps one question might be whether the life cycle itself is the reproducing entity. Godfrey-Smith (2013) disagrees, because there is clearly stage-to-stage causation of the process, which is not therefore best understood as a cause itself. This seems right. A different way to get rid of the notion of multigenerational reproduction is to divide cycles up into mere ‘successions’ of the same individual: a continuant (probably determined by genetic identity)

3. Herron et al. (2013) find that there are some organisms such as algae that have single-lineage bottlenecks but undergo germ–soma differentiation only in association with bacterial collectives. I address multilineal entities below.

that has different stages with connected succession. But in many protists, such as *Physarum*, 'stages' produce new generations and multiply the parent entity in different forms. The combination of strategies is distributed across the bigger unit—the multigenerational one. One explanation of such extended reproductive strategies is that much metazoan reproduction is a stripped-down simplified form of protist reproduction. For many large animals, everything happens in a linear process without subcycles, with clear phenotypic (and genetic) continuity between parent and offspring. Sex is obligatory, and 'asexual' reproduction occurs only to somatic cells. Somatic cells are abandoned at the bottleneck phase, and fitness is realigned for the next generation.

It is now common to discuss the ongoing evolution of eukaryotes as a history of not just complexification but also major simplification. There is a growing appreciation that the first eukaryote cells were hugely complex, both ultrastructurally and genomically, and that these original cells and genomes simplified as they diversified, with major eukaryote supergroups then adding innovations to those simplified cells and genomes (Wolf and Koonin 2013). Cytoskeletal components, crucial to reproductive machinery, have undergone secondary simplification in most major eukaryote groups (Yubuki and Leander 2013), thus suggesting that eukaryote reproduction has also evolved along similarly complex-to-simple lines.

In fact, given the limited diversity in metazoans, life cycles such as ours are best understood as nontypical of eukaryotes: we might indeed be "the odd ones out on the genetic playground" (Bracht et al. 2013, 406). Godfrey-Smith has suggested decreased reproductive complexity to be the case for metazoans: "What we think of as the machinery of reproduction in large familiar organisms is often the evolutionarily-compressed remnant of a much stranger lifecycle" (2009, 78). Eukaryote reproduction–nonreproduction cycles can be usefully conceptualized as conflict removal. It occurs via a more complex mechanism than the simple reduction of cell types and numbers known in animals. In fact, single-generation bottlenecks might be just a vestige of the earlier multigenerational process and thus belong to a larger class of phenomena. This class is what I am calling 'fitness alignment', a term commonly used about multispecies consortia and how they achieve mutualisms. But it is also applicable to multiple generations of the same lineage and a single generation of a lineage.

Thinking about how eukaryotic reproduction took a complex-to-simple evolutionary pathway has methodological consequences too. It suggests that there are limitations to taking humans and other animals as the model biological systems for anything, even if only eukaryotes are considered. A phylogenetically broad perspective of reproduction needs to include more complex reproductive modes. Extrapolation of what is typical for eukaryotes might, therefore, be done more successfully from protists than animals.

4. Multilineal Reproduction. Unicellular eukaryotes challenge standard concepts, but prokaryote reproduction goes further. Usually when single-cell prokaryote reproduction is discussed, the focus is binary fission, which occurs without meiosis or mitosis. My focus, however, is prokaryotes as reproducers forming multicellular collectives. Why? Because these collectives are intrinsic to major clade formations in evolutionary history, and they exhibit considerable continuity as functional multiplicative entities. I will make this case with the legume–rhizobia mutualism, which has persisted for 70 million years.⁴

The legume–rhizobia collaboration is a model system for the evolution of mutualisms. It involves initially free-living rhizobia. When they enter into alliances with legumes via the roots, the plants gain nitrogen, which is fixed by the rhizobial bacteria, and the bacteria gain carbon from the plant. Reproductive success increases on both sides of the symbiosis, and there is very little cheating (Friesen 2012). So far, so symbiotic. But this is a very curious relationship to be sustained for such a long time.

‘Rhizobia’ is a polyphyletic group (they can be genetically quite different), some of which are parasitic. Plants can be infected by multiple strains of the same rhizobial species. Rhizobia can be evicted from the plant roots or leave voluntarily, so they always have a free-living stage at some point in what may be many generations of bacteria. The plant itself experiences a brief early stage without symbionts, but to grow and reproduce, the plant must actively recruit them. The capacity to live apart and the ability to come together from environmental contact are both indicators of relationships that are arms races in exploitation and that should collapse owing to the many opportunities to cheat.

Fitness alignment seems to be the key to the persistence of such mutualisms. It is achieved by several mechanisms, such as sanctions, partner choice, and fidelity feedback (Friesen 2012). A relevant strategy is codevelopment, whereby bacteria and plant transform ontogenetically to maintain a series of checks and balances. It is not just a matter of the plant root developing in well-defined ways; the rhizobial bacteria develop too, into bacteroids, which are morphologically and intracellularly different from free-living rhizobia. Bacteroids develop with two membranes: one comes from the plant, and one is bacterial. This mutualistic codevelopment has gone on for many million years, even though rhizobia can and do live autonomously. The plant

4. Another powerful exemplar is the system comprising arbuscular mycorrhizal fungi, plants, and bacteria, which has reproduced itself for 450 million years (Kiers and van der Heijden 2006). The functional confluence of these genetically separate entities enabled the transition of plants to land and thus transformed the terrestrial environment while increasing oxygen levels in the atmosphere (Bonfante and Genre 2008).

can recruit different groups of organisms, but all recruits provide similar functions in the same collaborative arrangement. Fitness alignment is thus the default state of this liaison, and 'defector' rhizobia are sometimes theorized as merely 'defective' (Friesen 2012, 1104).

As was the case in unicellular eukaryotes, reconceptualizing development appears to go hand in hand with expanding the concept of reproduction. And codevelopment and coproduction of the relationship seem to be central to the maintenance and persistence of an evolutionary unit (Gilbert et al. 2010). Development functions to align the fitness and mutual interests of the symbionts and host. The various strategies deployed to maintain these alliances allow a different kind of reproducing multicellularity.

We might agree that such systems are empirically important, but not that they constitute any sort of theoretically relevant unit: only 'properly' reproducing entities fulfill this role. Multilineal systems do not because of the variable fidelity of the replicators involved. This exclusion ignores the fidelity of functional reproduction that allows the whole system to persist and regenerate over evolutionary time. Reproducing such systems involves a mix of vertical and lateral transmission strategies, with different components multiplying over different timescales. Perhaps even more so than the protists above, these systems exhibit very complex life cycles.

Why is there 'variable' fidelity of replicators? This is because transmission of symbionts can be vertical or horizontal. Parent-offspring lineages are formed by vertical transmission strategies, whether sexual or asexual. In the vertically transmitted symbiont case, symbionts are transmitted with host germ cells. The *Buchnera* symbionts of aphids are paradigmatic of this mode of transmission, which is almost always maternal (occasionally paternal or bi-parental). Vertical transmission is thought to ensure the fidelity of the mutualism via genetic restrictions. Although many symbionts may be transmitted with one egg, there are similarly constraining 'transmission bottlenecks' (Mira and Moran 2002). In nonvertically transmitted mutualisms, transfer of symbiont properties occurs horizontally from the environment. There is low replicator fidelity of parent-offspring relationships because symbionts may be genetically different even if they supply the same functions to the host. The legume-rhizobia system is a prime exemplar of this strategy.

Usually it is thought that horizontal transmission allows cheating, which leads to increased conflict and eventually mutualism breakdown. However, it is well known that obligate mutualisms persist despite horizontal acquisitions of symbionts, and that there may be good reasons for long-term mutualisms not to switch to higher genetic fidelity modes: "Combining vertical transmission with horizontal transmission greatly enlarges the range of conditions in which symbionts can persist and thus explains symbiont persistence" (Ebert 2013, 630). Mixed-mode transmission is therefore likely to be common, and strict verticality may often have horizontal contributions.

Mixed-mode systems include the combination of reproduction strategies in a host–symbiont system, such as in the legume–rhizobia mutualism. The plants themselves inherit characteristics vertically, whereas the symbionts are acquired repeatedly from the environment. Unlike the *Buchnera*–aphid system, this combination of vertical and horizontal strategies means that there are different evolutionary trajectories for the different components of the mutualisms. There are various ways this situation could be represented with regard to life cycles. One is to use diagrams that depict aposymbiotic (pre-symbiont) and symbiotic phases of a host–symbiont life cycle (Bright and Bulgheresi 2010; fig. 1).

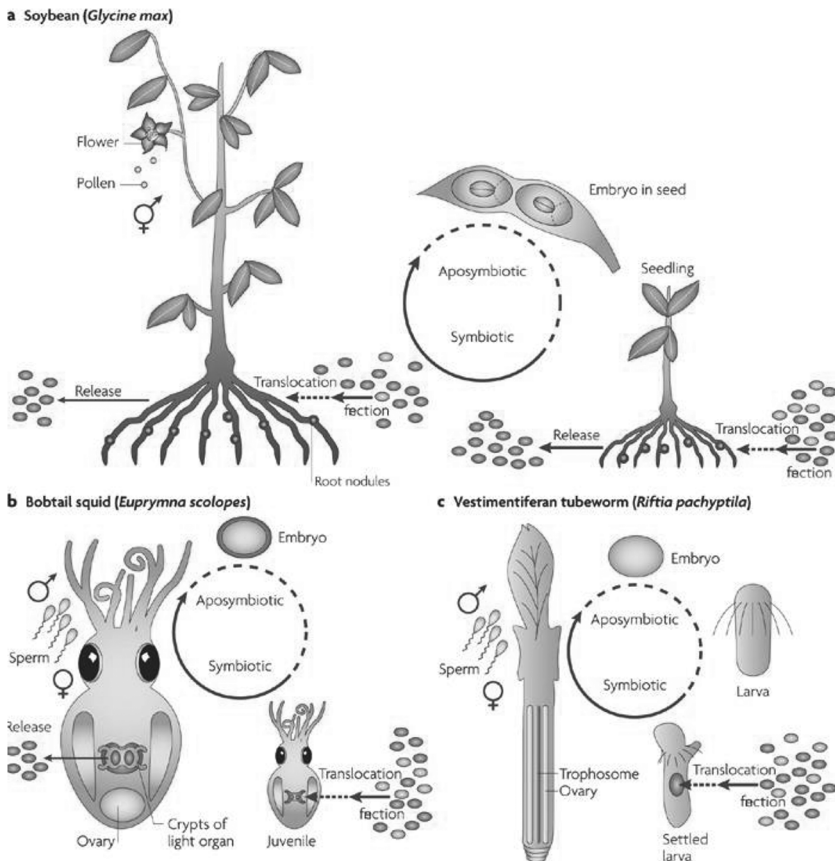


Figure 1. Life cycles with aposymbiotic and symbiotic phases (Bright and Bulgheresi 2010). *Dashed line*: aposymbiotic phase; *solid line*: symbiotic phase. Reprinted with permission from Macmillan Publishers Ltd.: *Nature Reviews Microbiology*, © 2010. Color version available as an online enhancement.

Host–symbiont life cycles with horizontal transmission are shown by a dashed aposymbiotic line, because the component organisms reproduce and can live separately for a time. The plants and symbionts discussed above clearly do this (see fig. 1*a*; Bright and Bulgheresi 2010), and it happens in animals too. Philosophers of biology often discuss the vibrio–squid unit (fig. 1*b*), but a tighter example is the *Riftia* tube worm found in hydrothermal vents (fig. 1*c*). Both types of organism composing this system exhibit major developmental reorganization in response to host–symbiont uptake. These tube worms at an immature stage have a basic digestive system (unlike most of their relatives), but they lose it as they mature. In fact, they give it up in order to gain all their metabolic and excretion capacities from occupying chemosynthesizing bacteria. As the bacteria enter the worms, both cocreate the specialized organ called the ‘trophosome’, where nutrient exchanges are carried out. Shorter aposymbiotic phases can occur with vertically transmitted episymbionts (outside the cell), which are common on animals such as worms (for details, see Bright and Bulgheresi 2010). In contrast, the vertical transmission of intracellular symbionts has no aposymbiotic phase, and this is where the aphid–*Buchnera* system fits.⁵

This more simplified and coordinated arrangement is the only one Godfrey-Smith considers as a Darwinian individual. Such combined life cycles are, however, more complex than just that of the host, even when the host is an animal with a standard germ line and bottleneck. For each generation of the host, there will be multiple generations of the symbionts. In many vertical transmissions, such as *Wolbachia* in insects, additional symbionts come in from the outside and supplement the *Wolbachia* already reproducing inside the host cells (Ebert 2013). In any host–symbiont system, therefore, mapping such relationships does not lead to tidy parent–offspring lines for the system as a whole.

In repeated horizontal transmissions, causal input from previous generations achieves continuity of function. That continuity is maintained generation after generation, despite genetic variability, because the capacity to align fitness is inherited (e.g., codevelopment). One response to functional continuity is to avoid the word ‘reproduction’ and use instead ‘mere recurrence’ (Godfrey-Smith 2013; De Monte and Rainey 2014). But doing so diminishes the distribution of reproductive mechanisms across these systems and how they achieve similar effects to those in standardly reproducing entities.

The mechanisms that enable functional fidelity in host–symbiont systems might be thought of as alternative modes of bottlenecking. As already dis-

5. The aphid lifecycle itself is quite peculiar, in that asexual mothers reproducing parthenogenetically and viviparously are ‘telescopically’ pregnant with daughters already pregnant with their own daughters (Davis 2012).

cussed, the idea of a bottleneck crucially includes the notion of genomes getting a ‘fresh start’ as the inevitable variation (and thus potential conflict) in somatic cells is eliminated. Something very similar occurs in these multi-lineal processes (which are also multigenerational from the perspective of the symbiont), as genetic variability is controlled for at the phenotypic level by processes that suppress conflict and enable the long-term survival and functional reproduction of the host–symbiont system.

Bottlenecks and vertical transmission in large animals can be regarded as special cases in a range of mechanisms by which form and function are reproduced over many generations. These specific mechanisms allow a certain kind of multicellularity (the kind we know as animal in particular), but not all multicellularity is of this type. In the examples above, the complex host–symbiont life cycles exist because of the co-option of symbionts for host function and vice versa, and the deployment of different mechanisms to align the fitness of the different organisms involved. One of the mechanisms for aligning fitness might be, in fact, the reversion to separate Darwinian individuals at aposymbiotic phases of the combined life cycle. Designating Darwinian individuals may indeed require distinct genetically continuous entities. But a mix of transmission strategies, within aposymbiotic and symbiotic phases of the life cycle, allows the overall ‘reproduction’ of equally distinct functional entities. Is this functional unit of evolutionary persistence a meaningful unit of evolution?

5. Units of Evolution and Fitness Alignment. To recapitulate: bottlenecks “de-Darwinize” (Godfrey-Smith 2009) groups of cells in standard multicellular entities by aligning fitness (ensuring that all cells are closely related and not selfish variants). Multigenerational and multilineal systems do not undergo this kind of de-Darwinization. Other mechanisms align fitness and ensure a certain kind of heritability (of functional properties), but they do not streamline reproduction. Are these larger units of reproduction Darwinian individuals, in the sense that fitness alignment “forces selection to act” on the collective (Clarke 2014, 310)? Probably not, but they are units of evolutionary persistence. The term ‘unit of evolution’, which is usually reserved for species, can also help identify entities that deploy reproductive strategies, inherit functional arrangements, form lineages, and have fitness aligned across them.

Why concede that these units of reproduction are not Darwinian individuals? Because Darwinian evolutionary theory is simply not targeting systems in which reproduction (or individuality) is distributed across generations and/or lineages. Their explanation requires additional conceptual machinery, but not the cancellation of Darwinian theory. One consequence of paying attention to such differently reproducing systems is that undistributed reproducers turn out to be ‘simple’ (e.g., metazoans), whereas distributed repro-

ducers (multigenerational protists, multilineage collectives) turn out to be the complex versions.

By combining different reproductive strategies that operate at different timescales, a whole functional unit is reproduced. For a protist, this unit is one in which different generations share reproductive tasks. In host–symbiont systems, multiple lineages use a variety of strategies to maintain collaboration across generations. Whether multigenerational or multilineal, fitness is aligned without standard bottlenecks between sexual generations, without germ lines of a restrictive sort, without simple parent–offspring continuity, and even without strictly vertical descent (although all those factors may apply to the isolated components of the larger unit). The examples I have discussed are units of reproduction, development, and evolution (or evolutionary persistence): they are the units across which biological individuality is distributed and fitness aligned generation after generation.

Including multigenerational protist life cycles and multilineal entities in discussions of reproduction results in a better understanding of how reproduction works and evolved, and generates insight into fitness alignment that goes beyond Darwinian individuals. This broader view achieves a novel focus on units of evolution and their capacities for persistence. In other words, Darwinian individuals are not all we should be interested in evolutionarily.

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