Rethinking evolutionary individuality

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This paper considers whether multispecies biofilms are evolutionary individuals. Numerous multispecies biofilms have characteristics associated with individuality, such as internal integrity, division of labor, coordination among parts, and heritable adaptive traits. However, such multispecies biofilms often fail standard reproductive criteria for individuality: they lack reproductive bottlenecks, are comprised of multiple species, do not form unified reproductive lineages, and fail to have a significant division of reproductive labor among their parts. If such biofilms are good candidates for evolutionary individuals, then evolutionary individuality is achieved through other means than frequently cited reproductive processes. The case of multispecies biofilms suggests that standard reproductive requirements placed on individuality should be reconsidered. More generally, the case of multispecies biofilms indicates that accounts of individuality that focus on single-species eukaryotes are too restrictive and that a pluralistic and open-ended account of evolutionary individuality is needed.

evolutionary individuality | microbial consortia | biofilms | reproduction | symbiosis

The word "individual" is ambiguous both in- and outside of biology. In biology there are genealogical individuals, such as species and phylogenetic taxa (1), metabolic individuals, those entities that use resources from the environment to maintain their structures (2), and individuals in natural selection, otherwise known as evolutionary individuals (3). In some cases, a biological entity is an individual of more than one type. A particular muskrat may be a metabolic individual and an individual in selection. In other cases, a biological entity may be just one type of individual. A species taxon is a genealogical individual but probably not an individual in selection. The focus of this paper is individuals in natural selection: that is, evolutionary individuals. Evolutionary individuals are those biological entities that satisfy Lewontin's three conditions for natural selection: they vary, that variation results in differential fitness among them, and that variation is heritable (4).

Biologists and philosophers of biology have recently suggested that some multispecies symbiotic consortia are good candidates for evolutionary individuals (5–9). Examples of such consortia include termite–fungi associations, aphid–bacteria consortia, and the human microbiome (10, 11). In this paper we focus on multispecies biofilm consortia and ask if they are evolutionary individuals. Not all multispecies biofilms are promising candidates for evolutionary individuals because of the amount of competition within them (6). Nevertheless, numerous types of multispecies biofilms, such as dental plaque and gastrointestinal consortia, have many features associated with evolutionary individuality. Such biofilms have internal integrity and are delineated from their environments. Their parts often coordinate their activities and in some situations cooperate. Furthermore, these biofilms have adaptive traits that are transmitted with fidelity between ancestral and descendant biofilms. However, such multispecies biofilms often violate standard reproductive criteria for evolutionary individuality: they lack reproductive bottlenecks and a significant division of reproductive labor, and they are composed of genomes from multiple species and those genomes do not form unified reproductive lineages.

What are we to make of such multispecies biofilms? If numerous multispecies biofilms are good candidates for evolutionary individuals, then standard reproductive requirements on individuality should be reconsidered. We do not deny that such criteria highlight important factors that contribute to the individuality of single-species eukaryote organisms. However, the case of multispecies biofilms (and other multispecies consortia) indicates that evolutionary individuality is achieved through other means than often-cited reproductive processes. We suggest that the existence of such multispecies individuals shows the need for a pluralistic and open-ended account of evolutionary individuality.

Biofilms and Evolutionary Individuality

We start with some biofilm biology. Biofilms are communities of bacteria, or communities of bacteria and other microorganisms. Some biofilms are made up of bacteria from one species, but most natural biofilms are comprised of multiple species (12, 13). How many species are found in multispecies biofilms? Algae-associated biofilms contain approximately 30 bacterial species (14). Dental plaque biofilms consist of hundreds of different bacterial species (13, 15).

Biofilms are thought to have life cycles and those cycles are typically broken into five stages (16–18), as follows. (*i*) The "planktonic lifestyle" is when the various bacteria that form a biofilm live separately (although this might better be thought of as a prebiofilm stage). (*ii*) "Attachment" is the initial attachment of bacteria to a surface. (*iii*) "Colonization" is the building of a biofilm through colonization and aggregation. Often this stage is broken into substages, with different bacterial species colonizing in sequential order. (*iv*) "Growth" namely means clonal production, which is typically regulated and coordinated through quorum sensing. Finally, (*v*) "dispersal": upon maturity a biofilm produces cells that are released to the environment.

During its growth stage, a biofilm produces an extracellular polymeric substance (EPS) that performs a number of functions (17, 19). A biofilm's EPS provides a biofilm with structural integrity. It also traps nutrients from the environment and contains enzymes that break those nutrients down for digestion by a biofilm's bacteria. EPSs even protect bacteria from threats, both predators and antibiotics, by providing a protective layer and by containing molecules that bind to antimicrobial agents that prevent them from attacking bacteria. Finally, EPSs serve as media for communication among bacteria through quorum sensing, and they foster the exchange of genetic material through lateral gene transfer.

Bacterial cells within biofilms use quorum sensing to coordinate a number of activities. Quorum sensing is based on the secretion

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and detection of molecules called "autoinducers." Bacteria use autoinducer concentrations as a proxy for population density within a biofilm. Bacteria tune their behavior according to the abundance of other cells in a biofilm. When concentrations of autoinducers within a biofilm reach a certain threshold, bacteria in that biofilm respond by altering their gene expression. For example, quorum sensing in *Pseudomonas aeruginosa* biofilms regulates the production of extracellular DNA that make up a biofilm's EPS (17). Such quorum sensing both induces the production of extracellular DNA to build an EPS, as well as downregulates extracellular DNA production to prevent the overproduction of an EPS, which wastes both energy and nutrients (17). Quorum sensing coordinates a wide range of behaviors within biofilms, including the production of EPS matrices (20), the secretion of chemicals that increases a biofilm's resistance to antibiotics (21), and the dispersal of cells from a mature biofilm (22). Quorum sensing is not restricted to members of the same species. Interspecific communication through quorum sensing also occurs in multispecies biofilms, such as oral biofilms (23) and pathogenic biofilms containing *P. aeruginosa* (24).

Another common feature of biofilms is lateral gene transfer. Two mechanisms are responsible for lateral gene exchange among the bacteria of a biofilm. Transformation is a mechanism that allows a bacterium to pull in extracellular DNA released from other cells (17). EPSs often contain large amounts of extracellular DNA (25). Conjugation is the transfer of mobile genes, such as plasmids, between bacteria through cell-to-cell bridges (26). How extensive is lateral gene exchange in biofilms compared with such exchange among planktonic bacteria? Watnick and Kolter (27) write that the bacteria of a biofilm "share their genetic material at high rates" compared with single bacteria. Hausner and Wuertz (28) report that rates of conjugal transfer are a thousand-fold higher in biofilms than in populations of planktonic bacteria. Nadell et al. (17) observe that "the closely packed environment in biofilms makes them ideal candidates for genetic exchange among cells."

Having introduced some biofilm biology, let us turn to some characteristics associated with evolutionary individuality that occur in biofilms. We suggest that because numerous multispecies biofilms have these characteristics, such biofilms are good candidates for evolutionary individuals. One commonly cited feature of evolutionary individuals is internal integration and delineation from the environment (29–31). Biofilms have this feature. The cells of a biofilm are bonded through aggregation and bounded by their EPS matrix. A biofilm's EPS keeps its parts together, helps defend a biofilm from external threats (predators and antimicrobial agents), and captures nutrients in the environment for its bacterial cells. Furthermore, bacteria within a biofilm are often highly integrated: they communicate, exchange genes, and "help" each other through a number of coordinated processes, from the production of their shared EPS to performing mutually beneficial metabolic processes (see below).

Two other often-cited markers of evolutionary individuality are division of labor and coordination among an individual's parts (29, 30, 32, 33). Examples of division of labor among the cells of a biofilm are plentiful. In some cases of biofilm formation, different colonizers perform different functions. The first colonizers of oral biofilms, such as *Streptococcus oralis* and *Escherichia coli*, produce adhesives for attaching to surfaces, whereas secondary colonizers, such as *Fusobacterium nucleatum*, serve as bridges between early and late colonizers that cannot bind to each other (12, 13, 34). Numerous mutualistic interactions involving chemical transformations also show there is division of labor within at least some biofilms. Biofilms are often spatially heterogeneous, with some bacteria occupying areas near the surface of a biofilm, other bacteria occurring in the middle of a biofilm, and others at further depths. Stewart and Franklin (35) report the case of biofilms consisting of surface bacteria that consume oxygen, intermediary bacteria that convert sulphide into hydrogen sulfate, and bacteria at greater depths that cycle sulfate into sulphide. (Further examples of such mutualist interactions are found in ref. 12.)

The above examples are of division of labor among the bacteria of a biofilm, but they are not obviously instances of coordination among those bacteria. Quorum sensing provides examples of such coordination. As we saw earlier, quorum sensing in *P. aeruginosa* biofilms regulates the amount of extracellular DNA bacteria produce for their mutual EPS. Another example is the release of autoinducers by one bacterial species that causes another bacterial species in the same biofilm to produce chemicals that render their biofilm more resistant to antibacterial agents (24, 36). Then there are cases of quorum sensing that cause certain cells within a biofilm to disperse (22).

Another indicator of evolutionary individuality is cooperation among the parts of an individual (3, 5, 37). Bacteria within biofilms frequently produce public goods. A public good is a cellular product that is costly to produce and enhances the fitness of other cells (38). Cheater bacteria within biofilms could receive the benefits of public goods without producing them. Cheating does frequently occur in biofilms; however, cheaters are often kept in check such that public good production is a common feature of biofilms. There are many examples of public goods in biofilms, such as the production of EPS compounds (17), antibiotic degradation compounds (38, 39), and denitrification processes (13). The production of public goods benefits both producers and neighbors. Could some bacteria in biofilms even be altruistic? Kreft (40) offers a model in which the strategy of low growth by individual bacteria yields higher total biofilm growth than the strategy of high growth by individual bacteria. Kreft suggests that this "altruistic strategy" "increases the fitness of the group by using resources economically at the cost of decreased fitness, or growth rate, of the individual" bacteria (40).

A question that immediately arises is how does cooperation among the cells of a biofilm persist? Why don't cheats spread throughout biofilms? Sometimes cheaters do get an upper hand in a biofilm. Nevertheless, the production of public goods is a common feature of biofilms. Why might that be? Researchers cite a number of mechanisms within biofilms that reinforce cooperation. Some multispecies biofilms are spatially structured such that cooperative bacteria occur in dense clusters insulated from noncooperative mutants that arise from bacterial strains in other portions of a biofilm (41). Another mechanism that promotes cooperation in biofilms is the lateral gene transfer of mobile genetic elements that infect noncooperative bacteria and cause them to produce a public good (42). Some instances of quorum sensing (43) and ecological disturbance (44) are also thought to keep cheaters in check. Cooperation within biofilms—and the mechanisms that enforce it—occur over and over again. This is not to say that cheaters never win. Nor is it to say cooperation occurs in all biofilms or even all of the parts of a biofilm. Bacterial cooperation within a biofilm is often imperfect and patchy (17, 45). Nevertheless, cooperation seems to be a reoccurring feature in some biofilms.

Another requirement placed on evolutionary individuals is that they are the bearers of adaptations (30, 37, 46). What is an adaptation and who is the bearer of an adaptation is a vexing issue. Folse and Roughgarden suggest that the bearer of an adaptation "must display adaptations at the level of the whole that are not present at the level of the components" (37). Biofilms appear to have biofilm-level adaptations that do not occur at the level of individual bacteria. Bacteria within multispecies biofilms are often more resistant to disinfection and antimicrobial agents than those bacteria living on their own or living in single-species biofilms (12, 24, 47, 48). Biofilms have various mechanisms that resist antibiotics that are not found at the level of individual bacteria. For example, EPSs reduce the penetration of antibiotic

molecules, and lateral gene transfer promotes the sharing of antibiotic resistant genes (17, 18, 26). More examples of biofilm level adaptations include metabolic interactions (12, 13), sequential aggregation (13, 49), and quorum sensing that induces higher antibacterial resistance (24). In all of these cases interactions among the bacteria of a biofilm increase a bacterium's fitness above what it would be if it lived alone.

A final ingredient for evolutionary individuality is the transmission of traits between generations of individuals (4, 31, 50). Microbiologists that study biofilms frequently talk about biofilm traits that occur over and over again. Many of those traits are discussed in this paper, such as quorum-sensing systems, metabolic interactions, aggregation patterns, cooperative behaviors, the mechanisms underlying lateral gene transfer, and the production of EPS components. Furthermore, specific genes within bacteria have been identified that transmit such traits between biofilms, such as genes underlying quorum-sensing mechanisms (23), genes underlying lateral gene-transfer mechanisms (51), genes that cause aggregation patterns (52), and genes that are instrumental in producing EPS components (53).

The claim that biofilm-level traits are transmitted is not controversial. What is particularly interesting about biofilms is how such transmission occurs. Typically, we think of evolutionary individuals transmitting their traits through reproduction. But, as we shall see, the case of biofilms raises questions about what sort of reproductive processes are necessary for evolutionary individuality, and even how we should conceive of reproduction.

Reproductive Criteria for Individuality

Numerous criteria for evolutionary individuality not yet canvassed focus on reproduction. According to standard theories of evolutionary individuality, individuals have reproductive bottlenecks, develop from single-species genomes, form unified reproductive lineages, and have a significant division of reproductive labor among their parts. Biofilms, as we shall see, often violate these reproductive criteria for evolutionary individuality. If numerous biofilms are good candidates for individuality because they display heritable biofilm level adaptations, then standard reproductive requirements placed on individuals should be reconsidered. We do not deny that such reproductive criteria highlight important factors that contribute to the individuality of single-species eukaryote individuals. However, we suggest that the case of multispecies biofilms shows that evolutionary individuality is achieved through other means than often-cited reproductive processes. In other words, the existence of such multispecies biofilms indicates the need for a pluralistic account of evolutionary individuality. Let us now turn to various reproductive criteria for evolutionary individuality and see how biofilms fare on those criteria.

A common criterion for evolutionary individuality is that individuals develop from single-species genomes (32, 54–56). There are two parts to this view: individuals develop from a single genome, and that genome belongs to one species. The underlying assumption behind this view is that genetic homogeneity among the cells of an individual diminishes competition among those cells (32). With the genetic homogeneity assumption comes the assumption that an evolutionary individual develops from the genome of one species, rather than being comprised of multiple species genomes. As Bouchard writes, "most definitions of individuals focus on single-species collectives" (57). Turning to biofilms, most natural biofilms violate this criterion for evolutionary individuality. As Elias and Banin (12) write, "[m]ixed-species biofilms are obviously the dominant form" (also see ref. 13). As we saw earlier, the number of species within a multispecies biofilm can range from a handful of species to hundreds of species. If biofilms are evolutionary individuals, then the genetic homogeneity requirement on individuality should be dropped.

The idea that there are multispecies evolutionary individuals is far from new. Biologists and philosophers of biology have recently focused on such multispecies evolutionary individuals. A frequently cited example is the symbiotic association between aphids and *Buchnera aphidicola*. A more complex example is the human-microorganism holobiont consisting of humans and their gut flora (9). Examples like these have caused some philosophers to reject the genetic homogeneity assumption of evolutionary individuality (7, 31, 58). Although philosophers of biology generally accept the existence of multispecies evolutionary individuals, they place constraints on such individuals. One constraint is that the different species lineages within those individuals run in tandem (2, 59). Godfrey-Smith (2) articulates this requirement using the example of the symbiotic relation between aphids and *B. aphidicola*. The parent–offspring lineages of aphids and the parent–offspring lineages of *Buchnera* bacteria run in tandem through vertical transmission. An aphid mother transfers bacteria to its offspring such that bacteria offspring are descendants of bacteria in parental aphids. Each aphid–bacteria multispecies individual, therefore, has a common beginning and ending, and those aphid–bacteria individuals form a unified reproductive lineage.

Do multispecies biofilms form such unified reproductive lineages? There are a couple of things to say here. First, in many cases the various species lineages that form a multispecies biofilm do not come together at the same time. Biofilm formation frequently occurs in stages. Some bacteria that form a biofilm are early colonizers, some are middle colonizers, and others are late colonizers. Biofilm formation, in other words, can be staggered. However, the fact that biofilm aggregation can be staggered does not stand in the way of such biofilms forming unified reproductive lineages. Like any fertilization or birthing process, biofilm formation can occur over time and have vague boundaries. However, on either end of that process (before aggregation starts and when it is completed) it is clear whether or not a biofilm is present.

There is another stumbling block to thinking that all biofilms form unified reproductive lineages: in many cases biofilms are formed by bacteria from multiple parental biofilms. Traditionally, we think of offspring having one or two parents. However, biofilms often have numerous parents: dozens if not hundreds. Nevertheless, that some biofilms have many biofilm parents does not show that such biofilms do not have the sort of parent–offspring relations required for individuality. We just need to think of the relations between parent and offspring biofilms in terms of complicated networks of numerous parents having numerous offspring. If packets of traits are faithfully transmitted between parent and offspring biofilms, then forming a unified reproductive lineage (in which the lineages that comprise a biofilm run in tandem) is not required for evolutionary individuality. This observation raises a general question about whether the production of new biofilms is reproduction. The answer to that question turns on how far one wants to expand the concept of reproduction from how it is traditionally conceived (we turn to this issue shortly). However, independent of how one defines "reproduction," many biofilms violate a reproductive criterion of evolutionary individuality: namely that the lineages of component species within multispecies individuals run in tandem.

Another reproductive criterion placed on evolutionary individuals is division of reproductive labor (31, 50). Mammals, for example, have distinct germ and soma lines. Only their germ-line cells can produce propagules that can then develop into descendent organisms. The distinction between germ and soma lines is thought to reduce competition among the parts of an individual because only mutations in germ-line cells can be passed on to future generations of organisms. Biofilms do not have specialized germ-line cells and lack any sort of germ–soma distinction. Nevertheless, some biofilms do exhibit a limited degree of reproductive specialization. In cases of swarming

dispersal, the cells released by a biofilm exhibit a specialized phenotype. The dispersal cells are motile, unlike most cells in the biofilm (16). So, although biofilms do not have a germ–soma distinction, some biofilms exhibit some division of reproductive labor later in their life cycles, during their dispersal stage. Still, that division of reproductive labor is far cry from the significant division of reproductive labor found in such organisms as mammals.

A different reproductive criterion placed on evolutionary individuals is having reproductive bottlenecks (31, 32, 56). A reproductive bottleneck occurs when an individual begins as a single cell (or a few cells). That cell is then replicated to form the cells of an individual. Reproductive bottlenecks are thought important for evolutionary individuality for two reasons. Bottlenecks reduce competition among the cells of an organism. As Maynard Smith and Szathmáry write, "the crucial reason why competition between the cells does not disrupt the organism is that, typically, development starts from a single cell, so that, apart from somatic mutation, the cells of an individual are genetically identical" (32). Another reason that reproductive bottlenecks are important for evolutionary individuality is their role in the evolution of new traits. As Godfrey-Smith observes, bottlenecks help the evolution of new traits in an individual because "a bottleneck forces the processes of growth and development to start anew, an initially localized mutation can have multiple downstream effects" (31). Any mutations in the germ-line of an individual that occur during its reproductive bottleneck are spread to its somatic cells.

Some biofilms may undergo reproductive bottlenecks, for example when a bit of a biofilm breaks off, drifts, and starts a new biofilm. However, typically biofilms form by aggregation, a process whereby numerous bacterial cells come together to form a new biofilm. Interestingly, biofilms have alternative processes that promote individuality along the lines that reproductive bottlenecks are thought to promote individuality. Biofilms can undergo ecological bottlenecks in which the population size of a biofilm decreases because of environmental factors, such as antimicrobial treatments (44). Similar to reproductive bottlenecks, ecological bottlenecks suppress conflict by increasing genetic relatedness among the cells in a biofilm. In addition, lateral gene transfer can spread evolutionary novelties within a biofilm without reproductive bottlenecks. Consider Ehrlich et al.'s (26) distributed genome hypothesis. That hypothesis is posited to explain the abundance of biofilms that cause chronic diseases. According to the distributed genome hypothesis, new disease strains among biofilms are a result of novel combinations of genes produced by lateral gene transfer. Moreover, laterally transferred genes encode key evolutionary traits for biofilms, such as antibiotic resistance (60) and the ability to produce public goods (42). Biofilms seem to achieve the outcomes of reproductive bottlenecks (genetic similarity and the transfer of novel mutations) by alternative means (ecological bottlenecks and lateral gene transfer). The existence of ecological bottlenecks and lateral gene transfer implies that reproductive bottlenecks may not be necessary for evolutionary individuality.

Stepping back from these details, we see that many multispecies biofilms score low on standard reproductive criteria for individuality: they don't form unified reproductive lineages, they don't have reproductive bottlenecks, and they lack a significant division of reproductive labor. Nevertheless, biofilms can achieve the results of reproductive bottlenecks by alternative means. In addition, biofilms are well-integrated units that are delineated from their surrounding environments. We have also seen that biofilms frequently consist of bacteria that coordinate their activities and in some cases cooperate. Finally, there are good reasons to think that biofilms pass on biofilm-level adaptations from generation to generation. These observations raise a general question. If numerous multispecies biofilms are good candidates for evolutionary individuals, then perhaps reproduction is unnecessary for evolutionary individuality? How one answers this question depends on how one defines "reproduction." If reproduction requires reproductive bottlenecks, then biofilms don't reproduce. If reproduction requires a significant division of reproductive labor, then biofilms don't reproduce. On the other hand, there are notions of reproduction that do not turn on bottlenecks or the germ–soma distinction.

Griesemer (61) offers an account of reproduction that requires neither bottlenecks nor a significant division of reproductive labor. According to Griesemer, reproduction is "multiplication with material overlap of mechanisms conferring the capacity to develop" (61). There are two parts to this account. Parents and offspring must have a genealogical relationship caused by material overlap, and entities capable of reproducing must have the capacity to develop or have life cycles. Biofilms satisfy Griesemer's account of reproduction. Once a biofilm matures, it releases cells to the environment. The released cells aggregate with other cells and form new biofilms. In some cases, though, bacterial cells multiply by binary fission during their planktonic stage. In those cases, it is the descendants of released cells from parental biofilms that aggregate to form new biofilms. Either way, there is a genealogical relationship between parent and offspring biofilms caused by material overlap. Furthermore, that material provides new biofilms with the capacity to develop. As we saw earlier, biofilms have various developmental stages in their life cycles: planktonic lifestyle, attachment, colonization, growth, and dispersal (16, 18).

Biofilms reproduce according to Griesemer's (61) account of reproduction. Does that mean that aggregation is a form of reproduction? We will not attempt to answer that question. What we will say is that if biofilms are evolutionary individuals, a choice must be made. Either some evolutionary individuals do not reproduce, or the notion of reproduction should be expanded to include at least some cases of aggregation. We should also mention that in discussing Griesemer's account of reproduction we are not advocating that it be adopted as the universal definition of "reproduction." It may leave out some reproducers, such as retroviruses (31). As we discuss below, trait transmission fidelity between individuals is essential for evolutionary individuality, not the particular mechanism that brings about that transmission. Perhaps some individuals successfully transmit their traits through reproduction involving bottlenecks. Perhaps other individuals successfully transmit their traits via processes best captured by Griesemer's (61) account of reproduction. The point here is that evolutionary individuals do not conform to one mode of trait transmission.

The Contingent and Pluralistic Basis of Evolutionary Individuality

The case of integrated multispecies biofilms should give us pause when it comes to standard reproductive requirements on evolutionary individuality. On the one hand, such biofilms have a number of features associated with evolutionary individuality. They have internal integrity and are delineated from their environments. They have biofilm-level adaptive traits that are faithfully transmitted between generations of biofilms. Furthermore, their parts coordinate their activities and frequently cooperate. On the other hand, such multispecies biofilms often violate standard reproductive criteria for evolutionary individuality. They lack reproductive bottlenecks and a significant division of reproductive labor. They are composed of genomes from multiple species and those genomes do not form lineages that run in tandem. If such multispecies biofilms are evolutionary individuals, then commonly cited reproductive requirements for evolutionary individuality are too restrictive.

However, one might object that the bacteria within a multispecies biofilm are more like members of an ecological community than parts of an individual. According to this objection, a biofilm is an adapted unit produced by selection acting separately on

each lineage within a biofilm. We disagree with this way of looking at highly integrated multispecies biofilms. Those biofilms are the foci of extensive lateral gene transfer, and such gene transfer undermines the assumption that biofilms are composed of independent lineages (62). Unlike multicellular organisms, the inheritance of genes in a biofilm is not confined to one species lineage but is distributed across different species within a biofilm. Consequently, genes within a biofilm are better conceived as public goods shared by different species (63). In addition to lateral gene transfer there are such synergistic interactions among the bacteria of biofilm as quorum sensing, mutualism, and cooperation. The bacteria of a biofilm do have distinct bodies. However, the connections and interpenetrations among those bodies go against the idea that those bacteria are in each other's environments.

Multispecies biofilms are not the only multispecies consortia that place a strain on often-cited reproductive requirements on individuality. Consider lichens, which are symbiotic consortia composed of fungal and photosynthetic partners (see ref. 6 for examples). Photosynthetic partners provide nutrients for their fungal partners, and fungal partners provide suitable living conditions (such as appropriate light intensity and moisture) for their photosynthetic partners (64). Despite their tight associations, lineages of fungal and photosynthetic partners do not always run in tandem. Some lichenized fungi recruit their photosynthetic partners from the environment (65). Lichens, thus, are similar to biofilms in important respects. On the one hand, lichens can be composed of separate species that form tight associations that occur again and again. Those associations carry adaptations that contribute to lichens' continued evolutionary success. On the other hand, lichens fail to satisfy standard criteria for reproduction because they are composed of independent lineages.

The existence of highly integrated multispecies biofilms and other consortia suggests that we need a more inclusive account of evolutionary individuality that allows individuals to have a variety of reproductive and trait transmission mechanisms. Furthermore, the existence of such multispecies consortia suggests that we need an account of individuality that is sufficiently open-ended to capture the contingent nature of individuality. Modes of reproduction and trait transmission are themselves products of evolution (66). A well-known biological fact is that evolution frequently produces different mechanisms that perform the same function. Think of the variety of mechanisms that allow organisms to propel themselves through water. Some organisms use a flattened tail, others use jet propulsion, and still others use their forelimbs or hindlimbs. Similarly, evolution is capable of producing different mechanisms for trait transmission and reproduction. Evolution has already produced a variety of such mechanisms. Those mechanisms include aggregation, reproductive bottlenecks, lateral gene transfer through conjugation, and lateral gene transfer via transformation. (For additional transmission and reproduction mechanisms in evolutionary individuals, see ref. 31.) Perhaps there are modes of trait transmission and reproduction not yet discovered. Perhaps new mechanisms for trait transmission and reproduction will evolve. Evolution is not over yet, and it would be unwise to bet against the evolution of new modes of trait transmission and reproduction. The case of multispecies biofilms and other consortia suggests that we need an account of individuality that is sufficiently inclusive and open-ended to capture the pluralistic and contingent nature of evolutionary individuality.

One might worry that adopting such an approach to evolutionary individuality is too loose and imprecise. When we suggest a more open approach to the reproduction and transmission mechanisms that underlie individuality, we are not recommending a conception of individuality that is completely open-ended. An account of evolutionary individuality should be constrained by Lewontin's (4) criteria for individuals in natural selection. According to those criteria, evolutionary individuals must exhibit variation that is heritable and results in differential fitness among individuals.

One approach to evolutionary individuality that decouples individuality from particular reproductive requirements is an interactor account of individuality. David Hull (30) introduced interactor theory in the 1980s. Numerous biologists and philosophers have advocated that approach to individuality, and it has been amended in various ways (3, 6, 62, 67). According to Hull, an interactor is "an entity that directly interacts as a cohesive whole with its environment in such a way that replication is differential" (30). By "cohesive whole" Hull means an entity whose parts interact (among themselves and with the environment) and that interaction causes the replicators of that entity (its genes or cells) to produce differentially. For example, quorum sensing and lateral gene transfer are two ways that cells within a biofilm interact. Such interaction affects the differential production of those cells, for as we have seen cells in biofilms often have higher survival and reproductive rates than lone bacterial cells.

Hull introduced the notion of interactor within an "interactor–replicator" framework for natural selection during the heyday of Dawkins' replicator theory (68). In that framework replicators "pass on their structures largely intact from generation to generation" (30). Replicator theory has been criticized. Griesemer (61) draws our attention to the fact that parents and offspring are often dissimilar, yet reproduction and trait transmission occurs. Godfrey-Smith (31) points out that although similarity in the traits transmitted is important, similarity among the mechanisms that do the transmission is not important. At this juncture, we want to suggest that an interactor account of evolutionary individuality need not be wedded to replicator theory. Perhaps in some cases trait transmission occurs in the form of replicators. In other cases such transmission fails to satisfy the requirements of replicator theory but occurs through traditionally recognized modes of reproduction, such as reproductive bottlenecks. In still other cases, transmission occurs in a fashion more in line with Griesemer's (61) account of reproducers. In addition, there may be forms of trait transmission that we have not yet discovered, and evolution may bring about new forms of trait transmission. As suggested earlier, the category of mechanisms that cause trait transmission should be open-ended to accommodate the pluralistic and contingent nature of evolutionary individuality. Interactor theory decouples evolutionary individuality from specific reproduction requirements. So interactor theory is appropriately open-ended and can capture the various types of evolutionary individuals that now exist and may exist in the future. We should hasten to add that interactor theory is not completely open-ended. Hull (30) developed interactor theory to satisfy Lewontin's (4) criteria for natural selection. Thus, interactor theory requires evolutionary individuals to have sufficient trait transmission fidelity for natural selection to occur.

The interactor approach to evolutionary individuality has much to commend it. Consider the possibility of evolutionary individuals nested in more inclusive individuals. The idea of nested evolutionary individuals is congenial to Lewontin's and Hull's accounts of selection (4, 30). Hull's and Lewontin's criteria for evolutionary individuality are not restricted to a particular level of biological organization. They allow that an evolutionary individual may be part of a more inclusive individual or that an evolutionary individual contain less inclusive individuals. Some multispecies biofilms arguably are evolutionary individuals nested within other evolutionary individuals. For example, multispecies biofilms found in our gastrointestinal tract display frequent lateral transfer of genes that encode key biofilm-level activities (69, 70). Such gastrointestinal biofilms are nested within more inclusive evolutionary individuals, namely mammalian organisms.

The case of biofilms and other multispecies consortia suggests that evolutionary individuals may be nested, and it suggests that being an evolutionary individual is not an all or nothing affair but comes in degrees. As we have seen, bacteria in multispecies biofilms often engage in synergetic interactions that benefit the different bacteria in a biofilm. Nevertheless, biofilms also often contain bacterial members whose interactions are antagonist. The fact that some biofilms exhibit more internal competition than others (17, 45) implies that some biofilms are more individual-like than other biofilms. Consider another case. Some biologists have hypothesized that eukaryotes, which are typically taken as paradigmatic evolutionary individuals, gradually evolved from symbiotic associations between eubacteria and archaea (71, 72). According to current biological knowledge, there is no sharp line in deep evolutionary history that distinguishes eukaryotes (paradigmatic evolutionary individuals) from symbiotic associations. Thus, among current multispecies consortia, as well as over time (during the evolution of eukaryotes), evolutionary individuality may be a biological characteristic that comes in degrees. The interactor approach to evolutionary individuality accommodates individuality being a matter of degree. Following Hull, there is a sliding scale concerning the degree to which the parts of an entity interact such that trait transmission to future generations is differential (30). The presence and strength of such synergistic interactions, such as quorum sensing, lateral gene transfer, cooperation, and mutualism, boost an entity's individuality. The lack of, or weakness in, such synergistic interactions along with antagonistic relations diminishes an entity's individuality. One might be uncomfortable with the vagueness of evolutionary individuality. However, the source of such vagueness may very well be the biological world rather than our theories about that world.

Another virtue of the interactor approach to evolutionary individuality is its decoupling of individuality from specific mechanisms of reproduction and trait transmission. As we have seen, the case of multispecies biofilms and other consortia suggests that evolutionary individuality may be achieved by various modes of reproduction and trait transmission. In eukaryotes such transmission is typically thought to occur via single-species lineages, through reproductive bottlenecks, and with a high division of reproductive labor. As suggested in this paper, some multispecies biofilms and consortia achieve evolutionary individuality but do not transmit their traits through single-species lineages. Nor do they have reproductive bottlenecks or a high division of reproductive labor. Trait transmission in such consortia is accomplished through both lateral and vertical gene transfer, and the reproduction (or production) of such consortia is typically accomplished by aggregation. The mechanisms by which evolutionary individuals transmit traits and reproduce (or produce) vary dramatically. Because the interactor approach to evolutionary individuality offered here is not coupled to a particular mode of trait transmission (although it is constrained by the requirement that such transmission have enough fidelity for selection to occur), it is sufficiently pluralistic to capture the various types of evolutionary individuality that evolution has produced. Moreover, it is sufficiently open-ended to capture the contingent nature of evolutionary individuality. One might be uncomfortable with an open-ended approach to evolutionary individuality. However, we believe that the need for such an approach stems from the ongoing and open-ended nature of evolution, not from conceptual imprecision.

Historically there has been a bias in the biological sciences that sees single-species eukaryote organisms as paradigm evolutionary individuals. That bias in no small part has been caused by our limited access to the microbial world. Not until recently, with the advent various genetic technologies, have we been able to gather vast quantities of data concerning microscopic organisms. Genomic studies of prokaryotes, of other microorganisms, and of microbial consortia provide an abundance of information about the microbial world. That information suggests that evolutionary individuals may be much more varied than we thought just 20 years ago. If multispecies biofilms and other consortia are evolutionary individuals, then we need an account of evolutionary individuality that properly captures those individuals. If microbial consortia are evolutionary individuals, then we may need to rethink what sorts of entities can be units of selection.

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