Biological Individuality: The Case of Biofilms[*]

Marc Ereshefsky and Makmiller Pedroso

Abstract: This paper examines David Hull's and Peter Godfrey-Smith's accounts of biological individuality using the case of biofilms. Biofilms fail standard criteria for individuality, such as having reproductive bottlenecks and forming parent-offspring lineages. Nevertheless, biofilms are good candidates for individuals. The nature of biofilms shows that Godfrey-Smith's account of individuality, with its reliance on reproduction, is too restrictive. Hull's interactor notion of individuality better captures biofilms, and we argue that it offers a better account of biological individuality. However, Hull's notion of interactor needs more precision. We suggest some ways to make Hull's notion of interactor and his account of individuality more precise. Generally, we maintain that biofilms are a good test case for theories of individuality, and a careful examination of biofilms furthers our understanding of biological individuality.

Keywords: Biofilms; Biological Individuality; Individuals; Interactors; Reproduction

There are many accounts of biological individuality (see Clarke 2010 for a survey). This paper focuses on two prominent accounts: David Hull's (1978, 1980) and Peter Godfrey-Smith's (2009, 2011a, 2011b, 2011c). We test those accounts of individuality using the case of biofilms. Biofilms are single or multispecies communities of microorganisms embedded in a self-produced extracellular substance. Biofilms are particularly useful for examining accounts of individuality

[*] *Preprint. Published in Biology & Philosophy.*

because they fail to satisfy common theories of biological individuality, such as having reproductive bottlenecks or forming parent-offspring lineages. Nevertheless, biofilms have many of the qualities required of individuals in natural selection. Biofilms have repeatable life cycles. They contain replicators (genes and cells) and a biofilm's interaction with the environment exerts a unitary effect on those replicators. The organisms of biofilms are distinct cells, yet the numerous ways those cells interact, and the transference of DNA among them, place a strain on the idea that the organisms in a biofilm are in each other's environment.

We think that biofilms are good candidates for biological individuals. Furthermore, we believe that the nature of biofilms has implications for Hull's and Godfrey-Smith's accounts of individuality. The nature of biofilms implies that Godfrey-Smith's account of individuality is too restrictive. Biofilms fare poorly on Godfrey-Smith's (2009) account of Darwinian individuals: biofilms fail to have reproductive bottlenecks; they do not stand in parent-offspring relations; and their reproductive division of labor is not high. Nevertheless, we suggest that biofilms are individuals. Hull's account of individuality is more inclusive than Godfrey-Smith's theory. Biofilms are not replicators, on Hull's account, but they are good candidates for interactors. Hull's account of individuality, however, would benefit from more precision. His definition of interactor turns on the notion of cohesive whole, and that notion is ambiguous and needs further specificity. In the end, we argue that despite the precision of Godfrey-Smith's account, Hull's notion of interactor provides a better basis for biological individuality. We conclude by suggesting some ways to make Hull's interactor account of individuality more precise. More generally, we maintain that biofilms are a good test case for theories of individuality, and a careful examination of biofilms furthers our understanding of biological individuality.

Biofilms

Before turning to Hull's and Godfrey-Smith's accounts of individuality we need to introduce our case study. Biofilms are single or multispecies communities of microorganisms. In this paper we focus on multispecies biofilms. Biofilms grow attached to a surface, embedded in a self-produced extracellular polymeric substance (EPS) (Hall-Stoodley et al. 2004). EPS matrices perform a number of functions (Flemming and Wingender 2010). Not only do they hold the cells of a biofilm together, EPS matrices serve as digestive systems: they accumulative nutrients from the environment and contain extracellular digestive enzymes. Furthermore, EPS matrices protect biofilms with molecules that bind to antimicrobial agents and prevent their access to biofilm cells. EPS matrices are also media for cell communication, through nitric oxide signalling and quorum sensing (see below), and EPS matrices foster the exchange of genetic material (see below).

A biofilm proceeds through a series of stages (Hall-Stoodley et al. 2004). Consider an oral biofilm. Its life cycle begins with surface attachment, for example, the adhesion of *Streptococcus gordonii* to tooth surfaces. After initial surface attachment, such biofilms form macrocolonies via clonal growth and aggregation. Secondary colonizers, such as *Porphyromonas gingivalis*, then coaggregate with the species already attached to tooth surfaces (Kolenbrander et al. 2002). Once the biofilm reaches maturation, dispersal cells are produced and released to the environment, completing the cycle. Generally, biofilm life cycles consist of four stages: planktonic lifestyle (cells grow as single unattached cells); attachment; colonization; and dispersal.

Various types of interactions within biofilms regulate biofilm life cycles. One type of

interaction is chemical signalling among the bacterial cells of a biofilm.[1] Consider nitric oxide (NO) signalling. Barraud et al. (2006) show that low concentrations of NO produced by *P. aeruginosa* trigger dispersion, and that a *P. aeruginosa* mutant lacking the enzyme for producing NO does not disperse. Another type of communication interaction is quorum sensing. Quorum sensing is a cell-to-cell signalling system that enables bacteria to respond to population density. Quorum sensing occurs through the secretion and detection of molecules called 'autoinducers.' When the concentration of autoinducers reaches a certain threshold, cell differentiation in a biofilm is affected (Davies et al. 1998). For example, high cell density detection via quorum sensing in *P. aeruginosa* activates EPS production (Sakuragi and Kolter 2007).

The organisms in biofilms also engage in mutualistic relations. Biofilms are spatially heterogeneous communities, and oxygen and nutrient concentrations decrease as depth in a biofilm increases (Stewart and Franklin 2008). Such spatial heterogeneity triggers mutualistic relations in multispecies biofilms. Stewart and Franklin (2008) discuss a biofilm containing aerobic heterotrophs, sulfate-reducing bacteria (SRBs), and sulphide-oxidizing bacteria. They write that "[i]n the surface layer of the biofilm, aerobic heterotrophs consume oxygen, and in the anoxic depths, SRBs produce hydrogen sulphide from sulphate. In an intermediary zone, where both sulphide and oxygen are present, sulphide-oxidizing bacteria recycle the sulphide to sulphate" (*ibid*.: 202). The actions of different organisms produce a beneficial environment for the entire biofilm.

The formation of multispecies biofilms occurs through coaggregation: "a process by which genetically distinct bacteria become attached to one another via specific molecules" (Rickard et al. 2003:94). Not every species in a biofilm can co-aggregate with each other. The species *Fusobacterium nucleatum* can co-aggregate with species that cannot bind to each other.

F. nucleatum thus acts as bridge between early and late colonizers that form oral biofilms (Hojo et al. 2009). In such cases, biofilm formation through coaggregation is a sequential process. Coaggregation is not only vital for biofilm formation, it is also essential for the existence of certain bacterial species. For instance, *Streptococcus oralis* and *Actinomyces naeslundii* grow when co-aggregated in locations where neither can grow alone (Palmer et al. 2001).

Another type of biofilm interaction is lateral gene transfer (LGT). LGT is gene transfer among bacterial cells that is not due to reproduction. In biofilms, LGT occurs among conspecific strains and stains in different species (Tibble et al. 2012, Wang et al. 2002). Biofilms provide favorable conditions for LGT. Consider two LGT mechanisms: transformation and conjugation. Transformation consists of the uptake of free DNA from the environment by a bacterial cell. Transformation requires extracellular DNA. In biofilms, this prerequisite is met because environmental DNA (eDNA) is a major constituent of biofilms. The other mechanism for LGT, conjugation, occurs via cell-to-cell junctions or bridges. Such bridges allow the transfer of mobile genetic elements, usually plasmids (Thomas and Nielsen 2005). The physical stability caused by EPS matrices reduces the chance of conjugal bridges breaking (Ehrlich et al. 2010: 273ff.). In short, lateral gene transfer occurs within biofilms for several reasons: the occurrence of extracellular DNA, high cell density, and the physical stability EPS matrices provide. Besides genetic exchange, LGT is also important in biofilm formation because conjugation plasmids induce planktonic bacteria to form biofilm communities (Ghigo 2001).

Stepping back from these details, we see that biofilms have repeatable life cycles. Various types of interactions within biofilms cause those cycles, such as quorum sensing, molecular signalling, coaggregation, and lateral gene transfer. Furthermore, EPS matrices serve as digestive systems, defence mechanisms, and media for communication. Biofilms are not mere

agglomerations of organisms, but groups of organisms with finely tuned interactions. Some biofilms consist of only conspecific organisms, but many biofilms consist of multiple species of organisms.

Godfrey-Smith's Darwinian Populations and Individuals

Godfrey-Smith (2009, 2011a, 2011b, 2011c) offers a comprehensive theory of natural selection that describes the types of populations and individuals (which he calls "Darwinian" populations and individuals) needed for natural selection. Godfrey-Smith's account starts with Lewontin's (1970) three conditions for natural selection: a population must have individuals that exhibit "variation in character, which leads to differences in reproductive output …, and which is inherited to some extent" (2009: 39). A welcome feature of Godfrey-Smith's account is that these three conditions are analyzed using parameters that vary in degree. For example, Darwinian populations can contain individuals with varying degrees of fidelity of inheritance (*H*). Using *H* and other parameters, Godfrey-Smith allows that Darwinian populations lie on a continuum between paradigm and marginal populations. Similarly, Darwinian individuals lie on a continuum between paradigm and marginal individuals. Paradigm Darwinian populations tend to produce complex adaptations, marginal Darwinian populations do not.

Lewontin's (1970) account takes reproduction as a given, and reproduction is central to Godfrey-Smith's account of individuality. However, it is well known that the notion of reproduction can be problematic. For example, what appears to be a grove of distinct aspen trees may be parts of a single organism growing from a common root system. To handle such cases, Godfrey-Smith (2009) distinguishes three types of reproducers: collective, simple, and scaffolded. Collective reproducers "are reproducing entities with parts that themselves have the

capacity to reproduce, where the parts do so largely through their own resources rather than through the coordinated activity of the whole" (*ibid*.: 87). Multicellular organisms, buffalo herds, colonies, and some symbiotic associations, are collective reproducers. The lowest-level entities capable of reproducing are simple reproducers. A simple reproducer has "the machinery of reproduction internal to it" (*ibid*.: 88**).** A bacterial cell is a simple reproducer. Scaffolded reproducers "are entities which get reproduced as part of the reproduction of some larger unit (a simple reproducer), or that are reproduced by some other entity" (*ibid*.). Chromosomes are scaffolded reproducers because chromosomes are copied by the cells that contain them.

To distinguish paradigmatic cases of collective reproduction from other such cases, Godfrey-Smith introduces three parameters: bottleneck (*B*), germ/soma distinction (*G*), and overall integration (*I*). We will discuss these parameters in detail, as they are central to Godfrey-Smith's account of individuality. We will see that the nature of biofilms places a strain on his account of individuality, particularly his account's emphasis on reproduction. The case of biofilms implies that reproduction plays a smaller role in biological individuality than often thought. We start with Godfrey-Smith's bottleneck parameter (*B*), and then turn to the parameters of germ/soma division (*G*) and overall integration (*I*).

Bottleneck

A number of authors cite the occurrence of reproductive bottlenecks as important for biological individuality (see Clarke 2010 for references). Godfrey-Smith defines bottleneck (*B*) as the degree of narrowing between generations. In cases of growth, there is no narrowing between generations and *B* is zero. In paradigmatic cases of reproduction, an adult develops from a small propagule, which produces a "narrowing" between generations (high *B*). *B* is zero when a new

structure is formed by the aggregation of cells, as opposed to being the result of cell divisions from a single propagule. As an example, Godfrey-Smith discusses the formation of fruiting bodies in *Dictyostelium*. *Dictyostelium* cells can behave as free-living entities during their feeding stage. When food is scarce and *Dictyostelium* cells begin to starve, they agglomerate, leading to the formation of "fruiting bodies" (Bracco et al. 2000). According to Godfrey-Smith, *Dictyostelium*'s fruiting bodies have no bottleneck because "[a] new fruiting body is formed by aggregation of many single-celled organisms; it does not grow by division from a small propagule" (2009: 95). Analogous reasoning applies to biofilms. Aggregation is a key process in the formation of biofilms. Consequently, biofilms have zero bottleneck.

It is useful to see why *B* is significant for Godfrey-Smith's account of individuality. High *B*, according to Godfrey-Smith, is important for the production of "evolutionary novelty" (2009: 91). *B* plays a crucial role in origin explanations (*ibid*.). Godfrey-Smith contrasts origin explanations with distribution explanations. In distribution explanations "we assume the existence of a set of variants in a population, and explain why they have the distribution they do or why their distribution has changed" (*ibid*.: 42). Distribution explanations do not explain how a set of variants originated. Origin explanations do. It is uncontroversial that natural selection offers a basis for distribution explanations. However, Godfrey-Smith suggests that natural selection also provides a basis for origin explanations.

> Mutation produces new genetic variants; but it produces them *from* pre-existing genotypes, and introduces them *into* a context comprising other genetic and phenotypic features. Those two facts are the key to the "creative" role of selection; selection shapes populations in such a way that combinations of genes and traits that are otherwise very unlikely

> to arise via the immediate sources of variation, become much more likely to arise. It does this by changing the population-level background against which new mutations appear. (*ibid*.: 49-50)

Godfrey-Smith believes that natural selection can provide origin explanations only if there are reproductive bottlenecks: "Because a bottleneck forces the process of growth and development to begin anew, an initially localized mutation can have a multitude of downstream effects" (*ibid*.: 91). According to Godfrey-Smith, the extreme narrowing between generations (high *B*) can alter the whole collective via mutations in the propagule, because the adult cells in a collective reproducer come from the same propagule. By fostering wholesale changes in a collective, bottlenecks can change "the population-level background against which new mutations appear**"** (*ibid*.: 50).

The absence of bottlenecks in collectives, according to Godfrey-Smith, decreases their potential for producing novel and complex adaptations. His argument for the link between bottleneck (*B*) and evolutionary novelty assumes that as *B* increases, the higher the chance that a mutation will have "downstream effects" within the rest of the collective. This argument assumes that gene exchange only occurs during reproduction. However, if genetic transfer can occur in a collective independently of reproduction, then a mutation can have "downstream effects" in a collective regardless of whether a bottleneck is present. In multicellular eukaryotes, gene exchange is typically associated with reproduction. In prokaryotes, genetic exchange and reproduction are decoupled. Prokaryotes exchange genes outside of reproduction via lateral gene transfer (Thomas and Nielsen 2005). Using the example of LGT in biofilms, we suggest that low bottleneck values (*B*) do not imply marginal individuality.

Biofilms provide a favorable environment for LGT. Biofilms contain the extracellular

DNA used in LGT by transformation, and EPS matrices increase the occurrence of conjugal bridges. Furthermore, microbiologists argue that LGT in biofilms is evolutionary significant (Langille et al. 2012). For instance, LGT is responsible for the transfer of antibiotic resistant genes (Davies and Davies 2010). Also consider the Distributed Genome Hypothesis (DGH) (Ehrlich et al. 2010). DGH explains the proclivity of biofilms that cause chronic diseases by citing the role of LGT within biofilms.

> [B]acterial biofilms associated with chronic infections are composed of multiple strains of a single species (as well as often being polymicrobial or polykingdom communities) and that real-time HGT [LGT] among the component strains (and species) leads to the continuous generation of a cloud of new strains with a novel combination of genes, thereby providing the bacterial community with a means to thwart the adaptive immune response of the host. (Ehrlich et al. 2010: 270)

According to DGH, biofilms associated with chronic diseases have a "population-based supragenome" that is larger than the genome of single strains within a biofilm. LGT continually rearranges the genes within a biofilm, contributing to the evolution of new strains. This reassortment causes the persistence of many chronic infections (*ibid*.: 276).

For Godfrey-Smith, high bottleneck, *B*, is required of paradigm Darwinian individuals because high bottleneck allows localized mutations (in a propagule) to be transmitted to the rest of the collective. By associating low *B* with more marginal cases of evolution, Godfrey-Smith's account implies that such transmission is not accomplished without bottlenecks. We contend that LGT in biofilms suggests otherwise. The example of DGH illustrates how LGT distributes genes in a collective with zero bottleneck. Moreover, that distribution increases the survivalship

of biofilms and their component organisms. The existence of zero bottleneck, thus, is not an indicator of marginal individuality.

Germ/soma distinction and overall integration

Godfrey-Smith uses bottleneck (*B*) to distinguish reproduction from growth. A further difficulty with reproduction is determining the level of reproduction. Does a buffalo herd reproduce, or do just the individual buffalos in a herd reproduce? To answer such questions, Godfrey-Smith introduces two other parameters: germ/soma distinction (*G*) and overall integration (*I*). *G* measures the degree of reproductive division of labor among a collective's parts (2009: 92). Godfrey-Smith allocates the highest *G* to humans (and other mammals) because of the sharp distinction between our germ and soma lineages. He assigns a zero *G* to sponges because any fragment of a sponge can give rise to a new sponge (*ibid*.). He assigns intermediary *G* to *Dictyostelium* (slime molds) because they have differentiated reproductive cells (2009: 95). During reproduction, some cells form stalks and others form spores (Bracco et al. 2000). Biofilms also have differentiated dispersal cells. In the case of seeding dispersal, only planktonic cells, as opposed to sessile cells that plant themselves on surfaces, give rise to new biofilms (Hall-Stoodley et al. 2004). Biofilms, like slime molds, have an intermediary reproductive division of labor (*G*).

Turning to the integration parameter *I*, Godfrey-Smith divides *I* into three components: division of labor (aside from *G*); maintenance of boundaries between individual and environment; and mutual dependence of parts with respect to viability. Intuitively, low *I* distinguishes reproductive collectives from aggregations of organisms. Buffalo herds have low *I*. Multicellular collectives, such as mammals, have high *I*. Sponges have intermediary *I* (Godfrey-

Smith 2009: 93-94).

How do biofilms score on *I*? There is significant division of labor among the parts of a biofilm. As discussed earlier, multispecies biofilms form mutualistic relationships. Different species within a biofilm detoxify different chemicals for other species in a biofilm (Stewart and Franklin 2008; Elias and Banin 2012). Another example of division of labor is the formation of biofilms through coaggregation. Some species are first colonizers, other species are secondary colonizers (Hall-Stoodley et al. 2004; Kolenbrander et al. 2002). Another measure of *I* is maintaining distinct boundaries between individuals and the environment. Biofilms, as we have seen, are distinct from their environment (Stewart and Franklin 2008). (Below we argue that it is problematic to say that a biofilm's organisms are in each other's environments.) Finally, the parts of many biofilms depend on each other for their viability. Bacterial cells typically have higher survivalship when they are part of a biofilm compared to when they are in their planktonic state (Costerton 2007). There are a number of reasons for such increased survivalship. EPS matrices protect bacteria with molecules that bind to antimicrobial agents and prevent them from entering bacteria (Flemming and Wingender 2010). EPS matrices also allow bacteria to withstand shear stresses in flowing environments such as rivers (Hall-Stoodley et al. 2004: 99). Lastly, biofilms offer efficient nutrient strategies for bacteria, such as catching nutrients and partially digesting them in a biofilm's EPS matrix (Flemming and Wingender 2010). Stepping back from these details, we see that biofilms have middling or higher *I*. Even though biofilms are not as integrated as mammalian organisms, biofilms have intermediate or higher values of *I*.

Individuals, organisms, and ecological communities

Thus far, our examination of Godfrey-Smith's account of individuals suggests the following. In

Godfrey-Smith's framework, paradigm individuals form parent-offspring lineages of organisms (high *G* and *I*) divided by bottlenecks (high *B*). Biofilms put a strain on this conception of individuality in two ways. First, LGT in biofilms shows that high or even middling bottleneck values are not required for non-marginal evolution. Biofilms have zero bottleneck values, but they have other mechanisms for transmitting genes within them. That transmission changes the genetic background for the occurrence and spread of evolutionary novelties. Second, biofilms have middling values for *G* and at least middling values for *I*. Nevertheless, biofilms do not stand in parent-offspring relations and their lineages spend part of their existence (their planktonic stages) apart. Given these facts, biofilms are not paradigmatic individuals on Godfrey-Smith's account. Moreover, given that they have zero bottleneck values, biofilms rate poorly as even middling individuals on Godfrey-Smith's account. Yet biofilms seem to perform the crucial functions needed for being individuals. They have repeatable life cycles and they are causally integrated wholes whose parts share genes. Furthermore, their interactions with the environment have relatively uniform effects on their constituents (more on this below).

Still one might object that the bacteria in a biofilm are more like members of an ecological community than parts of an individual. Indeed, Godfrey-Smith's *G* and *I* are designed to distinguish collective level reproduction "from mere lower level reproduction plus organization of the results" (Godfrey-Smith 2009: 94). However, we think that biofilms are closer to being individuals than ecological communities. We will illustrate this using an example from Sterelny's (2011) review of Godfrey-Smith (2009), namely Sterelny's example of the symbiotic association between ants and acacia trees. Acacia trees provide food and nest sites for ants; ants protect their trees from leaf cutting insects. For Sterelny, symbiotic consortia like the ant-acacia association are important "Darwinian regimes," though the ant-acacia associations are

not paradigm Darwinian individuals on Godfrey-Smith's account because "we still clearly have independent lineages" (*ibid*.: 494). Godfrey-Smith (2011a) agrees with Sterelny that ant-acacia alliances are significant cases of evolution by natural selection, and he agrees that ant-acacia associations are not paradigm individuals. For Godfrey-Smith, the ant-acacia association is an "adapted unit," but selection is not operating on this unit as a whole. Instead, selection is acting on two separate Darwinian populations, the ant population and the acacia population (Godfrey-Smith 2011a: 507).

Like Sterelny's ant-acacia example, biofilms are not single parent-offspring lineages. Applying Godfrey-Smith's reasoning to biofilms implies that such biofilms are merely "adapted units" due to selection acting independently on each lineage. On Godfrey-Smith's account, each species lineage within a biofilm "operates as part of the environment for the other" (2011a: 508). Biofilm are thus "co-evolving conglomerates" (*ibid*.). In Godfrey-Smith's framework, biofilms could only be (paradigm) individuals if they formed single parent-offspring lineages.

We think it is wrong to say that biofilms are co-evolving conglomerates of lineages in each other's environment. In a symbiotic ecological relation, such as between ants and acacias, component organisms maintain their bodily integrity. The organisms do not exchange genetic material, nor do they become molecularly bonded. Contrast this with the relations among the organisms within a biofilm. Though the organisms in a biofilm remain distinct cells, the various ways those cells are connected and the transference of DNA among them negates the idea that the organisms in a biofilm are in each other's environment. The organisms within a biofilm are connected in multiple ways. Through mechanisms that allow the formation of biofilms (coaggregation). Through mechanisms that allow biofilms to respond to population density (quorum sensing). And, through EPS matrices that protect biofilms from antibiotics and help

them digest nutrients. Then there is the sharing of DNA through LGT, and the sharing of conjugation plasmids that induces some bacteria to form biofilms (Ghigo 2001). The organisms of a biofilm do have distinct bodies. Yet the connections and interpenetrations among those bodies go against the idea that those organisms are in each other's environments. In sum, the multi-lineage nature of biofilms should not preclude biofilms from being individuals. The case of biofilms suggests that we should be more receptive to the idea of multi-lineage biological individuals.[2]

Godfrey-Smith (2011c) has recently considered the possibility of multi-species individuals. He cites the case of aphids and their symbiotic bacteria. Such symbionts have the same reproductive cycle as their host aphids: an aphid mother transfers bacteria to its offspring through its ovary. According to Godfrey-Smith, if we identify the individual with the aphid-bacteria combination, then such combinations stand in parent-offspring relations and form reproductive lineages. That sounds right. However, biofilms do not meet this criterion for individuality. The bacteria that form a biofilm are scattered in the environment and they come from different sources. Furthermore, their coaggregation occurs at different stages of biofilm formation. The bacteria of a biofilm do not stand in unified parent-offspring lineages because their lineages do not run in tandem. Consequently, biofilms do not form reproductive lineages. Godfrey-Smith's notion of a multi-species individual, thus, fails to capture biofilms. We would hasten to add that the occurrence of LGT in biofilms but not in aphid-symbiont combinations (Nikoh et al. 2010) arguably makes biofilms better candidates for biological individuals than aphid-symbiont combinations.

Godfrey-Smith (2011c) has also recently distinguished biological individuals from organisms. According to Godfrey-Smith, organisms are systems of entities that work together to

maintain a system's structure, and they do so by collectively using resources from the environment. Some organisms form reproductive lineages and are Darwinian individuals on Godfrey-Smith's schema, but some organisms do not form such lineages and are not Darwinian individuals. Godfrey-Smith cites the case of the Hawaiian bobtail squid that ingests bacteria that help the squid camouflage itself from predators. The bacteria, in turn, receive the benefit of a safe and nutritious environment within the squid. Even though such squids and bacteria have this symbiotic relation, Godfrey-Smith argues that the squid-bacteria combination is merely an organism (in his sense) and not a biological individual. His reasoning is that the squid-bacteria combination is "a metabolic knotting of reproductive lineages that remain distinct" (*ibid*.). The lineages remain distinct because the bacteria that are flushed in and out of the squids do not form a parent-offspring lineage that runs in tandem with the parent-offspring lineage of squids. (Contrast this case with the aphid-bacteria example of the last paragraph, where aphid and bacteria lineages run in tandem.)

A referee for this journal suggested that biofilms are organisms (in Godfrey-Smith's sense) and not individuals (on Godfrey-Smith's account or any reasonable account of individuality). Thus, biofilms are not counterexamples to Godfrey-Smith's theory of individuality. We disagree. Recall that Godfrey-Smith refers to the squid-bacteria combination as "a metabolic knotting of reproductive lineages that remain distinct." The bacteria that comprise a biofilm also remain distinct reproductive lineages. However, the knotting of a biofilm's bacterial lineages is more robust than the knotting of a squid and its bacteria. A squid's bacteria help camouflage the squid from predators; and the squid provides a nutritious and safe environment for its bacteria. By contrast, the bacteria within a biofilm share DNA through LGT. The bacteria of biofilms use quorum sensing to respond to population density, and they engage in

mutualistic interactions. Furthermore, the bacteria of biofilms produce common EPS matrices that protect them from antibiotics and help digestion. The interactions and interpenetrations among the bacteria of a biofilm far exceed the interactions among the members of the two partner symbiotic consortia often cited by philosophers of biology. Biofilms are a provocative case. They are neither paradigm nor middling individuals on Godfrey-Smith's account of individuality. Yet we contend, and argue further below, that they are good candidates for biological individuals.

Hull on Individuals, Replicators, and Interactors

We have seen that the example of biofilms shows that Godfrey-Smith's emphasis on reproduction is problematic. In particular, biofilms illustrate how two important roles Godfrey-Smith assigns to reproduction are satisfied without individuals standing in parent-offspring relations or having bottlenecks. First, LGT in biofilms distributes novel genes to other members of a biofilm without the occurrence of bottlenecks. Second, biofilms exhibit considerable integration (intermediate *G* values and at least intermediate values of *I*) without forming single parent-offspring lineages. Hull (1978, 1980) offers a more inclusive account of biological individuality, one that does not emphasize the existence of bottlenecks or reproductive lineages. Let us turn to Hull's account of individuality and see how it handles biofilms.

Hull does not offer one account of biological individuality but several. He offers his basic notion of individuality in his work on species (Hull 1976, 1978). Individuals must be spatiotemporally restricted entities. Hull also offers a two-fold account of individuality in his work on natural selection (Hull 1980). In that latter work, two different kinds of individuals are required for natural selection to occur: replicators and interactors. Replicators and interactors

must satisfy his basic criterion of individuality –they must be spatiotemporally restricted entities. In addition, replicators and interactors have their own specific criteria. In what follows, we introduce Hull's different notions of individuality and ask if biofilms are individuals in any of Hull's senses of individual. We argue that biofilms are interactors, and we suggest that thinking of individuals as interactors is a fruitful way to approach the individuality problem.

We begin with Hull's basic idea of individuality, the one found in his work on species (1976, 1978). There he draws the contrast between individuals and classes. Classes are groups of entities that can function in scientific laws. Such laws, on Hull's account, are true at any time and at any place in the universe. If 'All copper conducts electricity' is a law, then that law is true here and now, as well as 100,000 years ago on some distant planet. Copper is a class because samples of copper are spatiotemporally unrestricted –copper can occur anywhere in the universe. Individuals, unlike classes, consist of spatiotemporally restricted parts. The parts of an individual can only exist in a particular space-time region. Elephant parts are only parts of a particular elephant if they occupy a certain space-time region. Similarly, the populations of a species, though there might be spatial gaps among those populations, must occupy a particular space-time region, namely one where those populations trace back to a common ancestor.

Though spatiotemporal restrictedness is necessary for individuality it is, according to Hull, insufficient. "[I]ntegration by descent is only a necessary condition for individuality, it is not sufficient. If it were, all genes all organisms and all species would form but a single individual. A certain cohesiveness is also required" (1976: 183). All the members of a species are spatiotemporally restricted in that they all must be parts of a single genealogical lineage. However, on that notion of individuality, all of life is an individual, and we need a further requirement on individuality for species to be individuals. Hull suggests cohesiveness.

Unfortunately, the concept of cohesiveness is ambiguous in his work on species. Is a cohesive whole an entity whose parts causally interact in appropriate ways, or does a cohesive whole merely consist of parts that act in a unitary fashion? The latter sort of cohesion does not require causal interaction. In his work on species, Hull seems to adopt the latter notion of cohesion – where causal interaction is not required. He writes that the cohesion or evolutionary unity of a species lies in its organisms having "fairly consistent, recognizable phenotypes" (1978: 343). Furthermore, he suggests that such cohesion can be the result of processes that require the members of species to causally interact, or the result of processes that act independently on those organisms (1978: 343-344). Gene flow among the members of a species is an example of an interactive process among the members of a species. Genetic homeostasis and exposure to common selection regimes are examples of cohesifying mechanisms that act independently within or on members of a species. We will return to this ambiguity shortly. We will suggest that the parts of an individual should causally interact.

In his "Individuality and Selection" (1980), Hull offers an account of what sorts of individuals are necessary for natural selection. Minimally, he writes, selection can only act on spatiotemporally localized entities (1980: 313). However, more is needed. Specifically two types of individuals are required: replicators and interactors. Replicators "pass on their structure largely intact from generation to generation" (*ibid*.: 315). Hull suggests that genes and asexual organisms are replicators, but sexual organisms are not. An interactor is "an entity that directly interacts as a cohesive whole with its environment in such a way that replication is differential" (*ibid*.: 318). Hull suggests that organisms and colonies are interactors, but he is suspicious of more inclusive groups being interactors (*ibid*.: 325). Replicators and interactors are different types of individuals in natural selection, and both are needed for selection to occur (*ibid*., 318).

Some entities are both replicators and interactors, for example, genes (*ibid*.: 320, 325). Some entities are merely one type of individual.

Spatiotemporal boundaries and replicators

When we ask if biofilms are individuals we are interested in whether they are individuals in natural selection. This qualification is important, because determining whether an entity is an individual requires specifying the type of individual being investigated (see discussion below). Hull's minimal requirement for individuals in natural selection is their being spatiotemporally localized entities (1980: 313). Biofilms satisfy this requirement. A biofilm is a single or multispecies community embedded in a self-produced EPS. A bacterium cannot be part of a particular biofilm unless it is spatially connected to its biofilm's EPS matrix. Biofilms also have temporal boundaries because biofilms have life cycles with beginnings and endings (Hall-Stoodley et al. 2004). Recall that a biofilm begins with bacteria becoming attached to a surface and to each other. This can occur in stages, with first and then secondary colonizers (Kolenbrander et al. 2002). Once a biofilm reaches maturity, dispersal cells are produced and released to the environment. The spatial and temporal boundaries of a biofilm may be vague; nevertheless, biofilms are spatiotemporally restricted entities.

According to Hull, being a spatiotemporally restricted entity is necessary for being an individual in natural selection but it is not sufficient. Individuals must also be replicators or interactors, or both. Are biofilms replicators or interactors? Let us first look at whether biofilms are replicators. Replicators are necessary for selection because they ensure fidelity in inheritance (Dawkins 1982). Hull (1980: 315) writes that replicators must pass on their structure "largely intact." But how much of a structure needs to be passed on to be largely intact? According to

Hull, the entire genome of an asexual organism is a replicator because "[i]n asexual reproduction, the structure of the entire genome is transmitted" (Hull 1980: 321). By contrast, the structure of genomes in sexual organisms may be altered by recombination, so only portions of the genomes in sexual organisms are replicators.

What structural features of biofilms are replicated? Hull's discussion of colonies is helpful. Hull writes that colonies have the following features: "[t]heir boundaries are frequently distinct. They exhibit internal differentiation and division of labor. They have properties of their own – e.g. the percentage of organisms in each caste and the distribution of these castes throughout the colony" (1980: 322). Biofilms appear to have all of these features. An EPS matrix provides a boundary between a biofilm and its environment. In multispecies biofilms such as oral biofilms, there is division of labor, where one species benefits from the products of other species. Furthermore, coaggregation mechanisms restrict which species can be part of a biofilm. However, Hull thinks that replicators should also transmit their genetic features largely intact. Returning to his remarks on colonies, Hull writes that recombination is an obstacle for colonies being replicators: "sexual reproduction presents the same range of problems for colonies functioning as replicators as it does for organisms" (*ibid.*: 322). Although meiosis does not occur within biofilms, there is no guarantee biofilms will have the same genetic endowment every time they form. Not all strains of a biofilm's bacterial species make it into every instance of a biofilm (Kolenbrander et al. 2010: 478). Furthermore, according to the Distributed Genome Hypothesis, LGT "among the component strains (and species) [of a biofilm] leads to the continuous generation of a cloud of new strains with a novel combination of genes" (Ehrlich et al. 2010: 270). Given these considerations, it is doubtful that biofilms are replicators. Nevertheless, biofilms have constituent replicators, namely genes and bacterial cells.

Interactors

For Hull, fidelity in replication is insufficient for evolution by natural selection. Interaction with the environment is also required. Without interactors, the distribution of replicators cannot change via selection (*ibid*.: 320). Hull places two restrictions on interactors. First, an interactor must be a cohesive whole. Second, an interactor's interaction with the environment must have a unitary effect on its constituent replicators. Let us see whether biofilms satisfy these criteria.

Hull contrasts "cohesive wholes" with mere groups. Mere groups are spatiotemporally localized, but that is merely due to their being at the same location (*ibid*.: 314). Interactors should be more than a mere group because "[a mere] group can be selected only incidentally – e.g., because all its members happen to be in close proximity to each other" (*ibid*). Hull's talk of interactors being 'cohesive wholes' seems to imply that the parts of an interactor causally interact. Elsewhere Hull describes interactors as "functionally organized systems" and "organized wholes" (*ibid*: 325). He even writes that populations are interactors only if they have "populational adaptations, properties characteristic of the population as a whole that allow it to interact with the environment as a whole" (*ibid*). Talk of functional organization, organized wholes, and population-level properties seems to imply that interactors consist of causally interacting parts.

Biofilms satisfy this requirement for being an interactor. Biofilms are not mere groups of organisms that happen to be at the same location at the same time. Recall the various ways that the organisms of a biofilm causally interact. Quorum sensing is a cell-to-cell communication system that enables bacteria to respond to population density. *P. gingivalis*, for example, does not become part of an oral biofilm simply because it happens to be near other colonizers.

Appropriate molecular signalling through quorum sensing is required (Hojo et al. 2009). Another type of interaction that illustrates that biofilms are not mere groups is coaggregation. Not every species can bind to another through coaggregation. Coaggregation mechanisms specify which pairs of species can bind together (Rickard et al. 2003). Then there are mutualistic interactions among organisms in a biofilm that regulate its chemical environment, such as some species in a biofilm reducing oxygen levels while others turn sulphates into sulphide (Steward and Franklin 2008). Stepping back from these details, the orchestra of cell-to-cell interactions within a biofilm shows that biofilms are not mere groups but cohesive wholes *sensu* Hull.

Let us turn to Hull's second condition for an entity to be an interactor, namely that its interaction with the environment has a "unitary effect" on its constituent replicators. Hull describes this condition when discussing whether ecological communities are interactors. According to Hull, ecological communities are cohesive wholes, but "the effects of these interactions on [a community's] constituent replicators are not unitary" (1980: 327). To illustrate this, Hull compares organisms with ecological communities. On the one hand, an organism's interaction with its environment often affects the distribution of all of its genes. For instance, if an organism is malnourished and cannot reproduce, none of its genes are passed on to the next generation. On the other hand, the success or failure of the organisms within an ecological community can differ significantly. Though fates of the members of a community are interconnected, some members may thrive while others fare poorly.

How do biofilms score on this criterion for being an interactor? Does a biofilm's interaction with the environment have a unitary effect on its constituent replicators? If 'unitary effect' means that the failure or success of a biofilm affects the survivalship of its constituent cells (replicators) in a uniform way, then biofilms meet this condition. Bacterial cells typically

have higher survivalship when they are parts of a biofilm than when they are in their planktonic state (Costerton 2007). For instance, antibiotics that are effective against planktonic bacteria are less effective when they are part of a biofilm (Hall-Stoodley et al. 2004). There are several reasons why being a part of a biofilm increases a bacterium's survivalship. First, EPS matrices protect bacteria by containing molecules that bind to antimicrobial agents and prevent them from entering bacteria (Flemming and Wingender 2010). Second, biofilms allow bacteria to withstand shear stresses in flowing environments such as rivers (Hall-Stoodley et al. 2004: 99). Third, biofilms offer efficient nutrient strategies for bacteria. When starved, bacterial cells enter into a dormant mode of growth that leads to the production of small cells called "ultramicrobacteria" (UMB). UMB are non-adherent and tend to be metabolically dormant (Costerton 2007: 10ff.). When nutrients become available again, such cells mobilize and form multispecies biofilms suited to utilize nutrient resources. By forming biofilms, bacterial cells form "opportunistic self-mobilizing communities" capable of surviving environments, such as the deep ocean, that bacteria by themselves could not survive (*ibid*.: 64ff.). More generally, the capacity to form biofilms is central for explaining the proclivity of bacterial cells. Biofilms protect their cells from adverse environmental conditions and offer efficient strategies for nutrient consumption. A biofilm's interaction with the environment has a unitary effect on its bacterial cells.

One might object, however, that biofilms are no more interactors whose interactions have a unitary affect on their constituents than symbiotic consortia or ecological groups. We agree that symbiotic consortia and ecological groups have interactions that affect their constituents. In the squid-bacteria consortium discussed earlier, a selection force against the bacteria will negatively affect the bacteria and the squid that depend on those bacteria for camouflage. Similarly, selection that affects a segment of a buffalo herd and reduces the available gene pool

for the herd may decrease the fitness of all. Generally, the number of interactions a group experiences and the extent to which those interactions affects all the members of a group come in degrees. We contend that the interactions among the members of a biofilm and the degree to which they affect its members surpass the interactions among the members of an ecological group. As discussed throughout this paper, the bacteria of a biofilm have a robust number of interactions: from EPS formation to quorum sensing, from shared defensive and nutrient gathering mechanisms to the sharing of genetic material. The interactions and interpenetrations are more numerous then the examples of symbiotic interactions we have seen. Moreover, the sharing of genes through LGT demonstrates that the interaction among a biofilm's members has a more significant genetic downstream effect than found in symbiotic consortia or ecological groups. The interactive effects among the constituent replicators of a biofilm are far more uniform than similar effects among the replicators of an ecological group.

The upshot, then, is that biofilms are good candidates for being interactors. Consequently, they are individuals on Hull's selection account of individuality. Biofilms are individuals, despite their being multi-species individuals and despite not forming single parent-offspring lineages. Furthermore, biofilms are individuals even though they lack reproductive bottlenecks. Biofilms are individuals *qua* interactors, according to Hull's account of selection, yet they fail many common criteria for biological individuality.

Interactors and Individuality

We are sympathetic to Hull's interactor account of biological individuality. It more readily allows that biofilms are individuals in natural selection than Godfrey-Smith's theory. Dupré and O'Malley (2009) are also sympathetic to Hull's notion of interactor and adopt it in their

definition of life. However, they write that they will use Hull's notion of interactor "in a very different way than originally supposed by Hull. Interactors, in our view, are complex systems involving collaboration of many highly diverse lineage-forming entities. This sort of interactor, we suggest, is the most fundamental unit of selection" (Dupré and O'Malley 2009). Dupré and O'Malley's primary aim is to show that organisms like us are composed of multiple genetic lineages. For example, *Homo sapiens* is one lineage among numerous bacterial lineages that populate our bodies. This seems right. Our interest in this paper, however, is different. Our target is to argue that multi-lineage biofilms are individuals in natural selection. In that regard, Dupré and O'Malley's and our approach to individuality coincide: Hull's interactor account of individuality does a good job at capturing the variety of individuals in natural selection.

Nonetheless, we are not completely satisfied with Hull's account of interactors. We think it would benefit from more precision. In the remainder of this paper, we suggest ways to make it more precise. Such work involves tackling major issues in metaphysics and the philosophy of science, such as how to distinguish individuals from aggregates, and how to distinguish processes from pseudo-processes. These issues cannot be solved quickly. We will not attempt to solve them, but instead offer some directions for making Hull's notion of interactor more precise.

As a first stab, consider the following contrast. Individuals produce outcomes that are due to causal interactions among their parts. Non-individuals (that is, aggregates) do not produce outcomes due to the causal interactions among their constituents. A non-individual can fail to be an individual in two ways: it can fail to produce a relevant outcome; or if it does produce that outcome, that outcome may be due to the aggregated causal contributions of its constituents. This articulation of interactors has two parts. First, there is the general distinction between an

interaction causing an outcome versus an outcome being due to an aggregation of effects. Individuals, certainly biological individuals, should be distinct interacting wholes or distinct causal processes rather than aggregates or pseudo-processes. Salmon's (1978) example concerning processes and pseudo-processes helps illustrate this distinction. The interaction among the parts of a car is a process such that the car at time t affects the state of the car at time t+1. However, the shadow of a car on a road's railing is a mere pseudo-process: the shadow at time t does not affect the shadow at time t+1. The car is a continuous causal process, an individual. The shadow is an aggregate of numerous blockings of the sun by the car. This distinction is far from new. Some philosophers interested in the identity of individuals maintain that the identity of an individual depends on its parts being properly causally connected (Shoemaker 1979; Armstrong 1980).[3]

Of course, causal interaction among the constituents of a group does not make a group any kind of individual. The causal interaction among car parts does not make it a biological individual. It is an individual: an individual that is a car. Here we get to the second aspect of the interactor notion of individuality. When we ask if entities causally interact such that they are parts of an individual we need to specify the kind of individual we have in mind. We need to ask if those entities causally interact such that they produce the outcome required of the sort of individual in question. Bill Clinton and the Rock of Gibraltar exert gravitational forces upon one another. They form a gravitational individual. But that gravitational individual is not an individual in natural selection. This point is metaphysical and seemingly distant from questions about biological individuality. Yet it is relevant. When asking whether a group of organisms is an individual we need to specify the type of individual we are interested in. Otherwise, we will not know what type of interaction to look for.[4]

Framing the question of whether a group of entities is an individual in the above terms can help frame the debate over biological individuality. First, there is the question of what type of individual is at issue: is the entity in question an individual in selection, an individual in systematics, or something else? Keep in mind that being an individual of one type does not preclude an entity from being an individual of another type. Second, what type of interaction is required among the parts of the individual to produce the outcome required of the kind of individual in question? Third, do the entities in the group under consideration in fact causally interact to produce that outcome? (The tools mentioned in note 2 help answer this last question.)

Our disagreement with Godfrey-Smith is over the processes needed for a group of entities to be an individual in natural selection. (In other words, our disagreement occurs in step two of the three steps just mentioned.) We have suggested that biofilms consist of bacteria that causally interact such that biofilms are good candidates for individuals in natural selection. The bacteria within a biofilm replicate and proliferate in a unitary manner due to a number of causal interactions within biofilms: coaggregation for biofilm formation; EPS matrix formation for protection and nutrition; quorum-sensing and lateral gene transfer for cell differentiation; and mutualistic relations for detoxifying environments. Furthermore, if an important aspect of biological individuality, as Godfrey-Smith argues, is the transmission of mutations among the parts of an individual, then biofilms have that ability. In eukaryote organisms, reproductive bottlenecks are essential for spreading mutations to the parts of an individual. Biofilms do not have reproductive bottlenecks, but they spread their evolutionary novelties among component bacteria through lateral gene transfer. In brief, we have argued that biofilms have the sorts of interactive processes needed to be individuals in selection, despite their rating poorly on Godfrey-Smith's schema.

We do not claim to have provided a well-developed interactionist theory of individuality. That would require much more space than we have in this paper. What we have done is offer the example of biofilms to test Godfrey-Smith's and Hull's accounts of individuality. We have seen that the example of biofilms challenges Godfrey-Smith's account of biological individuality and favors Hull's interactor account. Despite the precision and care of Godfrey-Smith's account, we side with Hull's interactor model of individuality. We believe, however, that Hull's notion of interactor needs further refinement. We have suggested some ways it can be refined. Stepping back from these details, let us conclude with the following general observations. The concept of biological individuality is undoubtedly complex; and the nature of biofilms is confusing. Nevertheless, biofilms provide a good test case for accounts of individuality, and a careful examination of biofilms furthers our understanding of biological individuality.

Acknowledgments

We thank Matt Haber, Maureen O'Malley, Peter Godfrey-Smith, and three referees for this journal for their helpful suggestions. Thanks to Ford Doolittle and Conor Meehan for helping us learn about biofilms and microbial consortia. We also thank the participants at the Individuals Across the Sciences conference (Paris 2012) for their feedback and stimulating discussion. The Canadian Institutes of Health Research and the Social Sciences and Humanities Research Council of Canada provided financial support for this research.

References

Armstrong D (1980) Identity through time. In: Van Inwagen P (ed) Time and cause. D Reidel, Dordrecht, pp 67-78.

Barraud N, Hassett D, Hwang S, Rice S, Kjelleberg S, Webb J (2006) Involvement of nitric oxide in biofilm dispersal of *Pseudomonas aeruginosa*. J of Bacteriol 188:7344–7353.

Bracco E, Pergolizzi B, Peracino B, Ponte E, Balbo A, Mai A, Adriano C, Bozzaro S (2000) Cell signaling and adhesion in phagocytosis and early development of *Dictyostelium*. Int J of Dev Biol 4:733–742.

Brandon, R (1990) Adaptation and the environment. Princeton University Press, Princeton.

Clarke E (2010) The problem of biological individuality. Biol Theor 5:312-325.

Costerton J (2007) The biofilm primer. Springer, Berlin.

Davies D, Parsek M, Pearson J, Iglewski B, Costerton J, Greenberg E (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280:295–298.

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol and Mol Biol Rev 74:417–433.

Dawkins R (1982) The extended phenotype. Oxford University Press, Oxford.

Dupré M, O'Malley, M (2009) Varieties of living things: life at the intersection of lineage and metabolism. Philos and Theor in Biol. http://quod.lib.umich.edu/p/ptb/6959004.0001.003?rgn=main;view=fulltext

Ehrlich G, Ahmed A, Earl J, Hiller N., Costerton J, Stoodley P, Post C, DeMeo P, Hu F (2010) The distributed genome hypothesis as a rubric for understanding evolution in situ during chronic bacterial biofilm infectious processes. FEMS Immunol and Med Microbiol 59:269–279.

Elias S, Banin E (2012) Multi-species biofilms: living with friendly neighbors. FEMS Microbiol Rev, in press.

Flemming H, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633.

Ghigo J (2001) Natural conjugative plasmids induce bacterial biofilm development. Nature 412:442–445.

Godfrey-Smith P (2009) Darwinian populations and natural selection. Oxford University Press, Oxford.

Godfrey-Smith P (2011a) Agents and acacias: replies to Dennett, Sterelny, and Queller. Biol and Philos 26:501–515.

Godfrey-Smith P (2011b) Darwinian populations and transitions in individuality. In: B. Calcott and K. Sterelny (eds) The major transitions in evolution revisited. The MIT Press, Cambridge, pp. 65–81.

Godfrey-Smith P (2011c) The evolution of the individual. Lakatos Award Lecture, LSE, June 2011. http://www.petergodfreysmith.com/Evo_Ind_PGS_Lakatos_2011_Web.pdf

Hall-Stoodley L, Costerton J, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2:95–108.

Hojo K, Nagaoka S, Ohshima T, Maeda N (2009) Bacterial interactions in dental biofilm development. Crit Rev in Oral Biol and Med 11:982–990.

Hull D (1976) Are species individuals? Syst Zool 25:174-191.

Hull D (1978) A matter of individuality. Philos of Sci 45:335–360.

Hull D (1980) Individuality and selection. Ann Rev of Eco and Syst 11:311–332.

Kolenbrander P, Andersen R, Blehert D, England P, Foster J, Palmer R (2002) Communication among oral bacteria. Microbiol and Mol Biol Rev 66:486–505.

Kolenbrander P, Palmer R, Periasamy S, Jakubovics N (2010) Oral multispecies biofilm development and the key role of cell-cell distance. Nat Rev Microbiol 8:471–480.

Langille M, Meehan C, Beiko R (2012) Human microbiome: a genetic bazaar for microbes? Curr Biol 22: R20–R22.

Lewontin R (1970) The units of selection. Ann Rev of Eco and Syst 1:1–18.

Nikoh N, McCutcheon J, Kudo T, Miyagishima S-y, Moran N, Nakabachiet A (2010) Bacterial genes in the aphid genome: absence of functional gene transfer from *Buchnera* to its host. PLoS Genet. doi:10.1371/journal.pgen.1000827

Palmer R, Kazmerzak K, Hansen M, Kolenbrander P (2001) Mutualism versus independence: strategies of mixed-species oral biofilms in vitro using saliva as the sole nutrient source. Infect and Immun 69:5794–5804.

Rickard A, Gilbert P, High N, Kolenbrander P, Handley P (2003) Bacterial coaggregation: an integral process in the development of multi-species biofilms. TRENDS in Microbiol 11:94–100.

Sakuragi Y, Kolter R (2007) Quorum-sensing regulation of the biofilm matrix genes (pel) of *Pseudomonas aeruginosa*. J of Bacteriol 189:5383–5386.

Salmon W (1978) Why ask, 'Why?'? An Enquiry Concerning Scientific Explanation. Proc and Addr of the Am Philos Assoc 51: 683-705.

Salmon W (1984) Scientific explanation and the causal structure of the world. Princeton University Press, Princeton.

Shoemaker S (1979) Identity, Properties, and Causality. In: French P, Uehling T, Wettstein H (eds) Midwestern Studies in Philosophy VI. University of Minnesota Press, Minneapolis, pp. 321-342.

Sterelny K (2011) Darwinian spaces: Peter Godfrey-Smith on selection and evolution. Biol and Philos 26:489–500.

Stewart P, Franklin M (2008) Physiological heterogeneity in biofilms. Nat Rev Microbiol 6:199–210.

Thomas C, Nielsen K (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol 3:711–721.

Tribble G, Rigney T, Dao D, Wong C, Kerr J, Taylor B, Pacha S, Kaplan H (2012) Natural competence is a major mechanism for horizontal DNA transfer in the oral pathogen *Porphyromonas gingivalis*. MBio doi: 10.1128/mBio.00231-11.

Wang B, Chi B, Kuramitsu H (2002) Genetic exchange between *Treponema denticola* and *Streptococcus gordonii* in biofilms. Oral Microbiol and Immunol 17:108–112.

Wiggins, D (2001) Sameness and substance renewed. Cambridge University Press, Cambridge.

Notes

1. Though we often refer to the members of a biofilm as bacteria or bacterial cells, it should be noted that some biofilms consist of non bacterial microorganisms.

2. Here our work overlaps with the work of Dupré and O'Malley (2009). They suggest that "life…is typically found at the collaborate intersections of many lineages." Biofilms are an excellent example of this.

3. Here are two philosophical tools that help distinguish individuals from non-individuals. Reichenbach's notion of screening off helps determine whether an outcome is caused by an interaction among the parts of an individual or is the result of the aggregated effect of independent entities. If the interaction of entities screens off the aggregated effect of independent entities, then an outcome is due to interaction within an individual. See Salmon (1978, 1984) and Brandon (1990) for discussions of screening off. Another useful tool for distinguishing outcomes due to aggregation versus outcomes due to interaction is Salmon's (1978, 1984) mark transmission criterion. Using Salmon's car and shadow example, if a car is dented, that car will remain dented until it is fixed. The dent is a mark transmitted by the car, and the car is an individual or a process. If the shadow of the car cast on a road's railing changes because one segment of the railing is broken, that change (i.e., mark) is not transmitted to future instances of the shadow when the railing is not broken. Pseudo-processes do not transmit marks. The car's shadow over time is an aggregate of the car blocking the sun at different moments. It is a pseudo-process.

4. The idea that individuation can only occur when we specify the type of individual being individuated is a central tenet of the sortal view of identity (Wiggins 2001).