Inheritance by Recruitment:

A Reply to Clarke's "Levels of Selection in Biofilms"

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Abstract

Doolittle (2013) and Ereshefsky and Pedroso (2015) argue that selection can act at the level of biofilms and other microbial communities. Clarke (2016) is skeptical and argues that selection acts on microbial cells rather than microbial communities. Her main criticism is that biofilms lack one of the ingredients required for selection to operate: heritability. This paper replies to her concern by elaborating how biofilm-level traits can be inheritable

Keywords levels of selection – biological individuals – biofilms – aggregation – inheritance.

Lewontin (1970) describes natural selection as a process that operates whenever a population of individuals has three ingredients: variation, fitness differences, and heritability in fitness. He defines these three ingredients without specifying the mechanisms that produce them. The generality of Lewontin's account makes it applicable to different levels of biological organization, from molecules to communities. Following Lewontin's framework, Ereshefsky and Pedroso (2015) and Doolittle (2013) have claimed that some biofilms—and other microbial communities—exhibit inheritable variation in fitness and, consequently, are units of selection in Lewontin's sense. Clarke (2016) disagrees. Her main criticism is that biofilms lack one of the ingredients required for se-

lection to operate: heritability. This paper provides a reply to her criticism by further elaborating how biofilm-level traits can be inheritable.

Evolution produces a wide range of mechanisms of inheritance. For example, humans and other animals employ a curious system of inheritance where only half of the genes from each parent are transmitted to the next generation. In contrast, the parent and the offspring of certain stick insects look alike because they are clones of each other. In other cases, inheritance involves more than one species as in female aphids that vertically transmit their endosymbiont to their offspring, the *Buchnera aphidicola* bacterium, causing the parent-offspring lineages of aphids and their endosymbionts to run in tandem. Following Ereshefsky and Pedroso (2015), this paper argues that biofilms imply the theoretical possibility of an additional mechanism of inheritance. To use Doolittle's (2013) apt term, the behavior of some biofilms suggests that inheritance can be achieved via *recruitment*.

A major obstacle in understanding how biofilms can exhibit inheritance is that they are partially formed by 'aggregation,' a process in which different cells from the environment are recruited to be part of a biofilm. One of the consequences of aggregation is that it increases genetic variation within a biofilm relative to multicellular organisms that develop from a zygote or other single-celled stage. At first glance, aggregation seems to bar inheritance at the biofilm level because if aggregation constantly changes the genetic makeup of biofilms, biofilm-level phenotypes would be too short-lived for natural selection to operate. This is one of Clarke's objections:

> [T]he species composition of the community can change over time in an open-ended fashion as its metabolic capacities change, and as interactions between species change, suggesting that gene frequency change occurring within the 'lifetime' of a biofilm will swamp any

between-biofilm effects (p. 200).

Nevertheless, one of the main points made by Ereshefsky and Pedroso (2013, 2015) was that aggregation in biofilms is *not* "open-ended." In particular, multispecies biofilms can have co-aggregation mechanisms that determine which species can aggregate together (Rickard et al., 2003). A biofilm does not simply recruit any microbe that happens to be in its vicinity. Moreover, coaggregation mechanisms can partly explain why mature biofilms exhibit stable phenotypes, such as the ability of oral biofilms to cause caries (which will be further discussed below).

Clarke claims, however, that co-aggregation mechanisms are not sufficient to ensure inheritance at the biofilm-level:

> [T]hey [mechanisms of co-aggregation] cannot ensure that all of the species from the 'parent' biofilm make it into the 'offspring' (Kolebrander et al. 2010). So there is reason to doubt that biofilms are able to pass *genes* on to successive generations with sufficient reliability for competition between biofilms to produce any response (emphasis added, p. 200)

Co-aggregation *does* allow biofilms to change their species composition across generations. For instance, the species *Fusobacterium nucleatum* in oral biofilms can co-aggregate with different types of oral bacteria. Accordingly, even if the species *F. nucleatum* is present in a biofilm, its presence does not guarantee that this biofilm will be composed of the same type of bacteria—and, consequently, by the same genes—as a preceding biofilm. As sexual organisms illustrate, however, changes in gene frequency across generations do not prevent an offspring from resembling its parents. Sexual organisms can even be the product of a mix of genetically different cells, such as fraternal marmoset twins that exchange cells while in the uterus (Godfrey-Smith, 2009). Hence, changes in gene frequency in

successive biofilms do not necessarily preclude biofilm-level inheritance if such changes remain within certain bounds (cf. Clarke (2016), fn. 6).[1]

Some biofilm phenotypes are quite resilient to changes at the genetic level. Consider, for example, the ability of oral biofilms to cause caries or periodontitis:

> [T]he absence of *S. mutans* does not ensure caries-free dentition. Single organisms can be reduced in number with little change in outcome for the host because the vacated niche is filled by another bacterium (or group of bacteria) with similar functionality in pathogenesis. Thus, the relationship of various bacterial physiologies to one another, and the overall functionality (caries inducing and periodontitis inducing) created by the community, are important for disease development (Kolenbrander et al., 2010, p. 478).

Similar to biofilms, other microbial communities, such as gut microbiomes (Turnbaugh et al., 2008) and lichens (Dal Grande et al., 2012), can preserve their overall function despite some variation in their genetic makeup. As Doolittle points out, these examples indicate that heritability in biofilms and other microbial communities can occur via recruitment:

> So comparable microbial ecosystems are alike because they independently *recruited* functionally similar organisms, not because they have *inherited* their microbiotas from each other or some common source (Doolittle, 2013, p. 366).

Recruitment is an evolutionary mechanism by which successive biofilms can resemble each other. Furthermore, it does not require the same genes to be passed on because similar functions (e.g., the ability to produce a substrate) can be encoded by different genes.

[1] This is not to say that biofilms have the same heritability as sexual organisms. My main point is that aggregation does not automatically preclude biofilm-level inheritance.

Clarke states that inheritance by recruitment posits a 'non-genetic source' of inheritance. However, inheritance by recruitment is still *genetic* inheritance.[2] Co-aggregation is a genetically controlled mechanism that utilizes phenotypes to determine which strains will be part of a biofilm (Reardon-Robinson et al., 2014). Moreover, recruitment keeps genetic variation within the bounds required for biofilms to look like each other. An oral biofilm may not be composed of *S. mutans* but this does not mean that any species—and, consequently, any gene—can fill the vacant niche. Accordingly, an oral biofilm can still cause caries in the absence of *S. mutans*.

In another type of objection, Clarke grants that there are similarities between biofilms but she disagrees that these similarities are due to inheritance at the biofilm level. More specifically, Clarke proposes the following dilemma:

> [T]hanks to the way that combinations of microbes are reshuffled between biofilm generations, fitness-enhancing novelties will either lose their fitness effect entirely when they are transmitted to offspring, or they are context-independent and so better conceived as cellular traits (p. 201).

Clarke's dilemma relies on a particular recipe for identifying biofilm-level adaptations. According to her, the function of a biofilm-level adaptation should depend on the particular species or strains present in a biofilm; i.e., the function of a biofilm-level adaptation should be "context-dependent." She explains her reasoning in the quote:

> [I]f we find that ECM [extracellular matrix] production is triggered by one lineage whenever it finds itself in the company of *any* other strain or species—if the trait is context-independent, in other words—

[2] This is not intended to be a statement of Doolittle's (2013) position. For Doolittle, inheritance in multispecies consortia can be due to a process akin to cultural evolution.

> then it makes more sense to view ECM production as a competitive adaptation *of that cell lineage* (emphasis added, p. 201).

According to Clarke's dilemma, a biofilm trait is either context-dependent or context-independent. A context-independent trait is not a biofilm-level adaptation but a trait of one of its cell lineages. If a biofilm-level trait is context-dependent, this trait will probably be lost in the next generation given how much a biofilm's structure changes in composition over time. This leads Clarke to conclude that "[w]hole biofilms only exhibit heritable traits if their component lineages migrate collectively to new niches, and we do not see this happening" (p. 201).

Biofilms have context-dependent traits that can be inherited via recruitment because traits of different cell lineages within a biofilm are tuned to interact with each other. For instance, mixed biofilms in the lungs of cystic fibrosis patients containing the species *Pseudomonas aeruginosa* and *Burkholderia cepacia* can be more virulent than biofilms that only harbor *P. aeruginosa*. The higher virulence of the mixed biofilm is probably due to communication between these two species (McKenney, Brown, and Allison, 1995). *P. aeruginosa* and *B. cepacia* employ a cell-cell signalling system called 'quorum-sensing' to collectively express certain biofilm-level phenotypes, such as the production of extracellular matrix and the secretion of virulence factors (Williams et al., 2007). *P. aeruginosa* and *B. cepacia* use the same chemical language, the AHL-based quorum sensing system, allowing these two species to communicate with each other and increase their virulence when coaggregated (Riedel et al., 2001). Furthermore, genome analysis of *P. aeruginosa* isolates suggests that these two species exchange genes horizontally, placing a strain on the view that the *P. aeruginosa* and *B. cepacia* lineages are independent from each other (Eberl and Tümmler, 2004). The *P. aeruginosa*-*B. cepacia* consortium illustrates that cells in biofilms

can have context-dependent traits that are not lost in successive generations.[3] Aggregation may cause biofilms to be more genetically variable than mammalian organisms, but this does not mean that biofilm phenotypes are short-lived.[4]

Another point Clarke makes is that we could explain biofilm-level traits by appealing to selection on cells. For instance, some biofilm traits could be understood as the product of ecological succession:

> We can view a cell that leaves a biofilm as a propagule that develops into an offspring biofilm, or we can view it as a migrating individual that acts as a founder of a new ecosystem, without changing any empirical data (p. 197).

Even if the evolution of certain biofilm traits can be understood as a case of ecological succession, this does not change the fact that some biofilms can exhibit inheritable variation. The main point of this reply is that aggregation does not necessarily prevent biofilms from being individuals of selection in Lewontin's sense.

Acknowledgments: I am indebted to Marc Ereshefsky for his encouragement and suggestions. I would also like to thank Ellen Clarke, Ford Doolittle and the DC/Maryland History and Philosophy of Biology discussion group for their valuable feedback.

[3] See Elias and Banin (2012) for further examples.

[4] One might find it instructive to contrast multispecies biofilms formed by aggregation to multispecies consortia in which the partners are transmitted vertically, such as the aphid-*B. aphidicola* consortium (see above). In the case of vertical transmission, the partners tend to evolve traits that are specific to a particular partner (e.g., gene loss in *B. aphidicola*). Species in biofilms are expected to be more generalist than species acquired vertically because the same species may interact with different species. Yet, coaggregation mechanisms foster a certain level of specialization because the range of partners that a species in a biofilm is expected to interact with is limited. I thank Clarke for bringing this point up.

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