The Impact of Population Bottlenecks on the Social Lives of Microbes

Makmiller Pedroso
mpedroso@towson.edu
Philosophy & Religious Studies
Towson University, USA

Abstract

Microbes often live in association with dense multicellular aggregates, especially biofilms, and the construction of these aggregates typically requires microbial cells to produce public goods, such as enzymes and signalling molecules. Public good producers are, in turn, vulnerable to exploitation by free-rider cells that consume the public goods without paying for their production costs. The cell population of a biofilm or other microbial aggregates are expected to pass through bottlenecks due to a wide range of factors, such as antibiotic treatments and dispersal. The goal of this article is to make the case for the relevance of population bottlenecks at shaping the social interactions within microbial aggregates. The effect of bottlenecks on microbial aggregates is complex in that bottlenecks can favor producers under certain circumstances, but not in others. The concept of Volunteer's Dilemma from game theory will be used to motivate the hypothesis that this partly occurs because of how bottlenecks alter the risk of being a producer in a microbial aggregate. Finally, the role of bottlenecks in the microbial world impacts key issues in evolutionary biology, including the importance of ecology at shaping social evolution, and the evolution of multicellularity from unicellular ancestors.

Keywords: microbes – biofilms – public goods – Volunteer's Dilemma – bottlenecks – cooperation – free-riding.

1 Introduction

The bacterium species *Pseudomonas aeruginosa* is infamous for causing persistent infections in cystic fibrosis patients (Cystic Fibrosis Foundation, 2014). The

tenacity of *P. aeruginosa* infections is largely due to its cells forming densely packed groups called "biofilms" that attach to the lungs of cystic fibrosis patients (Fux et al., 2005). Like *P. aeruginosa*, a wide range of microbes can aggregate and form dense multicellular aggregates, such as the slugs built by the amoeba *Dictyostelium discoideum* and spore formation by the myxobacterium *Myxococcus xanthus* (Crespi, 2001). Accordingly, living in association with dense multicellular aggregates is an integral part of the microbial lifestyle (Hall-Stoodley et al., 2004; Claessen et al., 2014; Flemming et al., 2016; Stacy et al., 2016).[1]

The multicellular aggregates built by microbes often undergo population bottlenecks, that is, drastic reductions in population size. In particular, population bottlenecks can occur at different stages of a biofilm life-cycle. Biofilms are typically founded by small surface-attached colonies (Stoodley et al., 2002). For example, studies using enamel chips in humans reveal that the formation of dental plaque, a type of biofilm, starts as sparse aggregates of cells (Palmer et al., 2003; Kolenbrander et al., 2010). Biofilms can even be founded by single cells, as observed by the colonization of *Vibrio cholerae* of the small intestine in infant mice (Millet et al., 2014). Moreover, during their lifetime, biofilms face disturbance events that could reduce the size of an aggregate because of a series of factors, such as antibiotics (Anderl et al., 2000; Nickel et al., 1985; Stewart and Costerton, 2001), UV radiation (Elasri and Miller, 1999), and protozoan predators (Matz and Kjelleberg, 2005). Population bottlenecks can also occur after a biofilm matures. In particular, clumps of cells can shed from the biofilm due to mechanical processes, such as fluid shear (Stoodley et al., 2001; Hall-Stoodley et al., 2004). Finally, bottlenecks are expected to occur when pathogens infect a new organism due to host defenses and resource limitation (Abel et al., 2015). Different lines of evidence thus suggest that population bottlenecks are common in the microbial world.[2]

[1] Microbes can form multicellular structures in different ways (Claessen et al., 2014). Cells might fail to completely separate after cell division as in the case of filamentous cyanobacteria. Alternatively, microbial clusters can be partially formed via the aggregation of different cells as illustrated by biofilms and slime molds. The expression 'microbial aggregate' is being used to emphasize that this article focuses on microbial groups formed via aggregation.

[2] As the examples in this paragraph illustrate, the narrowing due to a bottleneck event is a matter of degree in the sense that a bottleneck can reduce a population to a single cell (e.g., *Vibrio cholerae* infections) or to multiple cells (e.g., clumping dispersal in biofilms). For this reason, the experiments discussed in this article often describe bottlenecks in relation to a reference population; that is, as the fraction of cells from a reference population that managed to pass through the bottleneck. Moreover, as it will be discussed in the next section, the degree of narrowing due to a bottleneck is crucial for determining whether cooperators or free-riders will be favored.

Studying the effect of population bottlenecks in microbial aggregates can impact foundational issues in social evolution. Population bottlenecks illustrate how feedback loops between ecological dynamics and evolutionary dynamics can affect social evolution (Post and Palkovacs, 2009; Moreno-Fenoll et al., 2017; Sanchez and Gore, 2013). More specifically, bottleneck events might partly explain why genes that encode social traits may exhibit high variability (Greig and Travisano, 2004; Dimitriu et al., 2014), why cooperators and free-riders often coexist within microbial groups (Archetti and Scheuring, 2011; Damore and Gore, 2012), and how the transition to multicellularity might have evolved (van Gestel and Tarnita, 2017). Finally, given that many pathogens live in dense multicellular aggregates (Hall-Stoodley et al., 2004), understanding the social interactions within such aggregates might enable the more efficient targeting of unfriendly microbes (Boyle et al., 2013).

This article is divided into three sections. The first section makes the case for the relevance of population bottlenecks in shaping social interactions within microbial cell aggregates. Experiments involving different types of microbes indicate that bottlenecks can affect the level of cooperation between cells (Brockhurst, 2007; Greig and Travisano, 2004; Chuang et al., 2009), and exert lasting effects in the evolution of microbial cell aggregates (van Gestel et al., 2014; Kreft, 2004; Dai et al., 2012; Vogwill et al., 2016). The second section is more speculative and suggests a partial account of how population bottlenecks could affect the level of cooperation within microbial aggregates. The concept of Volunteer's Dilemma from game theory (Archetti, 2009a; Diekmann, 1985) will be used to motivate the hypothesis that population bottlenecks can alter the risk of being a cooperator. According to this hypothesis, when the size of an aggregate decreases because of a bottleneck, cooperators may be favored because not as many cells can afford to free-ride on other cells without causing the aggregate to break apart. However, if the reduction in the aggregate size is too extreme, cells have an incentive to free-ride because living in a multicellular aggregate is no longer beneficial. The final section summarizes some of the main points of this article.

2 How population bottlenecks impact social evolution

2.1 Population bottlenecks in the lab

Microbial consortia such as *P. aeruginosa* biofilms are well-known for their persistence and for their ability to reoccur in different environments (Costerton et al., 1999; Folkesson et al., 2012). The resilience of microbial aggregates is enhanced by the ability of their cells to produce public goods, such as enzymes and signalling molecules, which are costly to produce but enhance the fitness of neighboring cells (Hall-Stoodley et al., 2004; De Kievit and Iglewski, 2000; Greig and Travisano, 2004). However, the production of public goods is vulnerable to exploitation by free-riders, i.e., cells that consume the public goods without paying for their production costs (West et al., 2007). A well-known system that illustrates the social conflict between producers and free-riders is the production of adhesive polymers by the bacterium *Pseudomonas fluorescens* (Rainey and Rainey, 2003; MacLean et al., 2004). When growing in a glass of broth, *P. fluorescens* cells produce a polymer that allow them to stick together and form biofilms at the air-broth interface which improves their access to oxygen. This polymer is a public good: its production has a metabolic cost for producer cells, but it generates a collective benefit (i.e., better access to oxygen). However, a strain of *P. fluorescens* can evolve from producer cells through mutations that can inhabit a biofilm without producing the costly polymer. The cells in this strain are free-riders: they reap the benefits of being part of a biofilm without paying the construction costs.

In order to examine how population bottlenecks affect social evolution, Brockhurst et al. (2007) subjected *P. fluorescens* cells to periodic disturbances (the system was disturbed every one, two, four, and eight days over a 16 day period). The disturbance treatments were designed to emulate mass-mortality events that bacteria face in the wild, and they consisted of discarding about 99.9% of the population each time ($\sim 10^6$ cells remained after each treatment). Accordingly, disturbances in this experiment were artificial population bottlenecks. It was observed that as the disturbance frequency increased, the proportion of producers increased to a certain point; past this point, increasing the disturbance frequency caused the proportion of producers to diminish. In short, Brockhurst et al. (2007) observed that the frequency of cooperators in *P. fluorescens* biofilms peaked at an intermediate disturbance frequency (i.e., one bottleneck

every four days). Further experiments illustrated that population bottlenecks can affect the level of cooperation of different species of bacteria, including *P. aeruginosa* (Ross-Gillespie et al., 2009) and *Escherichia coli* (Chuang et al., 2009).

In addition to bacteria, the relevance of population bottlenecks to social evolution has also been observed in the budding yeast *Saccharomyces cerevisiae*. Yeast cells digest sucrose outside of the cell by secreting the enzyme invertase. Like the adhesive polymer produced by *P. fluorescens* cells, invertase is a public good: producer cells have a lower growth rate relative to nonproducers cells, and approximately 99% of the digested sugars diffuse away from the producer cells (Gore et al., 2009). This makes a group of invertase producers vulnerable to exploitation by free-rider cells that consume the digested sugars while producing little or no invertase (Greig and Travisano, 2004). In well-mixed populations of producers and free-riders, it was observed that cell density can significantly affect the relative fitness of producers (Sanchez and Gore, 2013).

Population bottlenecks are an integral part of the microbial lifestyle because of how microbial aggregates are formed and the fact that microbes often live in harsh environments. The laboratory experiments discussed in this section provide evidence that population bottlenecks can affect the level of cooperation in eukaryotes and bacteria. The next section takes a closer look at the potential limitations of the reviewed laboratory experiments on population bottlenecks.

2.2 The utility and limitations of studying bottlenecks in lab settings

The insights generated by the experiments cited in the previous section are largely due to the fact that the effect of population bottlenecks were measured under artificial conditions. This allowed different variables to be manipulated, such as the the frequency and the degree of narrowing due to bottleneck events. However, the simplifications made by these experiments are also limiting in that they open the possibility that the effect of population bottlenecks on social evolution might be less pronounced under more natural settings.

It should be noted, however, that the laboratory experiments vary in how contrived they are. Specifically, a series of laboratory experiments have studied the effect of population bottlenecks under different ecological circumstances. The effect of population bottleneck on the level of cooperation in *P. fluorescens* biofilms reported in Brockhurst et al. (2007) persists when food supply is manip-

ulated (Brockhurst et al., 2010), and when *P. fluorescens* cells grow in heterogeneous environments (Buckling et al., 2000). Similarly, in yeast populations, population bottlenecks can favor cooperators (invertase producers) when the population is structured in such a way that cooperators remain closer together (MacLean et al., 2010), or when population bottlenecks are a result of competition with another species (Celiker and Gore, 2012). Accordingly, there exists evidence that population bottlenecks might affect social interactions across different ecological settings.

Yet, there is still the question of how the conditions in the laboratory experiments compare with the conditions microbes face in the wild. Studying bottlenecks in the wild is challenging partly because of how difficult it is to specify when, where, and how a bottleneck event occurred. Nevertheless, different methods have been used to study bottlenecks in more natural settings, including the introduction of genetic markers to infer the size of bottlenecks after they occurred (Abel et al., 2015), and the direct monitoring of bacteria during infection (Millet et al., 2014). Still, laboratory studies are particularly instructive for identifying ecological variables that might modulate the impact of population bottlenecks in the wild. The reviewed experiments reveal that the effect of bottlenecks on social evolution depends upon different factors, including the size and the frequency of bottleneck events. Moreover, they show that the effect of population bottlenecks on social evolution is also complex in that bottlenecks can favor producers under certain circumstances but not in others. In short, laboratory experiments illustrate the different ways bottlenecks can influence social interactions among microbes, but further empirical work would be required for a broader understanding of how bottlenecks impact microbial populations in more natural settings.

2.3 Population bottlenecks in a broader context

Different lines of evidence indicate that environmental disturbances can affect the propensity of organisms to form social groups (Bourke, 2011, ch. 4). For example, a comparative study involving distinct species of starlings shows that cooperative breeding is positively correlated with semi-arid savanna habitats and environments with temporally variable rainfall (Rubenstein and Lovette, 2007). As Rubenstein (2011) suggests, cooperative breeding in starlings might be a risk-averse strategy that reduces variance in fecundity induced by environmental fluctuations. In the case of microbes, frequent fluctuations in the

environment have been observed to select for *Vibrio cholerae* cells that are flexible strategists in the sense that they can switch between forming biofilms and living as free-swimming cells (Yan et al., 2017). Population bottlenecks in microbes are often caused by environmental disturbances, such as predation and antibiotic treatments. As a result, population bottlenecks illustrate a particular process through which environmental disturbances can alter the level of cooperation between organisms.[3]

Understanding how microbial aggregates respond to bottlenecks could help explain the early stages in the evolution of multicellularity (Libby and Rainey, 2013; van Gestel and Tarnita, 2017; Ratcliff et al., 2017). The initial evolution of multicellularity had to overcome a social challenge: multicellular individuals, including multicellular organisms and microbial aggregates, could only evolve if they were capable of suppressing the selfish interests of their cells. As the evolution of cancer illustrates, the reproductive interests of multicellular individuals and their cells do not have to always align with each other. Still, the evolution of multicellularity requires the presence of mechanisms that can limit the amount of genetic conflict within multicellular individuals (Rainey and De Monte, 2014).

Although multicellularity evolved more than 20 times from unicellular ancestors, the life cycle of multiple examples of multicellularity contain a single-cell bottleneck (Grosberg and Strathmann, 1998). The ubiquity of one-cell stages is striking because this particular type of life cycle is especially vulnerable to disturbances, such as predation and environmental fluctuations. Upon closer inspection, however, the persistence of unicellular stages is expected since single-cell bottlenecks can reduce the chance of conflict within individuals by increasing the genetic relatedness among their cells (Maynard Smith and Szathmáry, 1995; Godfrey-Smith, 2009). In other words, single-cell bottlenecks cause the reproductive interests of an individual and its cells to align with each other because most cells in the individual share the same genes. But how did unicellular stages first evolve?

Studying the effect of bottlenecks on multicellular aggregates provides some clues of the initial evolution of single-cell stages. In particular, Pichugin et al. (2017) have recently proposed a model to study the adaptive value of different ways groups may fragment into smaller groups, including division into equal size groups and single-cell bottlenecks. The study indicated that groups of

[3]In the case of environmental fluctuations that involve bottleneck events, bet-hedging might evolve more easily because bottlenecks can reduce competition between genetically related cells (Libby and Rainey, 2011; Beaumont et al., 2009).

cells that undergo single-cell bottlenecks maximize population growth when the benefits associated with group living only manifest when the group is sufficiently large. In their view, "when there is little gain until group size is large, it makes sense to maintain one group that reaps this advantage" (Pichugin et al., 2017, p. 15). In a related study, Ratcliff et al. (2013) used the unicellular green alga *Chlamydomonas reinhardtii* to investigate the initial evolution of single-cell bottlenecks. The authors reported the *de novo* evolution of multicellular clusters with a unicellular stage after subjecting *C. reinhardtii* populations to conditions that favor the evolution of multicellularity. One of the main results of this study was that unicellular bottlenecks conferred fitness benefits even in the absence of conflict among *C. reinhardtii* cells. Accordingly, the studies by Pichugin et al. (2017) and Ratcliff et al. (2013) suggest that unicellular stages might have initially evolved because of their selective advantage. The current ability of single-cell bottlenecks to limit genetic conflict would have been a by-product of selection at the cellular level.

Undergoing unicellular bottlenecks is not the only way individuals can avert the risk of subversion from within. In some animals and algae, the evolution of germ-soma specialization is also responsible for reducing genetic conflict among cells (Buss, 1987; Hanschen et al., 2017). Population bottlenecks could have facilitated the evolution of sterile soma cells if, as proposed by Nedelcu and Michod (2006), adaptive responses to stress were co-opted in the multicellular state to produce sterile soma cells. Overall reproductive success can involve a trade-off between two components of fitness: survival and reproduction. At first, stress due to population bottlenecks would have reorganized fitness at the collective level by taking resources away from reproduction and allocating them to survival (Michod and Nedelcu, 2003; Michod, 2005). For example, if cells need to live in dense cell aggregates in order to survive certain harsh conditions, selection might favor cells that sacrifice their own growth rate to help the collective—e.g., by producing a public good—in order to ensure the aggregate will not break apart (Archetti, 2009a). The evolved adaptations that initially allowed cells to cope with stress would later be co-opted to create soma cells specialized in survival instead of reproduction (Grochau-Wright et al., 2017). Accordingly, the traits that led to the evolution of soma cells could have been 'exaptations' (Gould and Vrba, 1982) that initially evolved to cope with the stress posed by population bottlenecks.[4]

[4]One related issue is the question of how natural selection should be conceptualized in order to capture cases of social groups in which parent-offspring relations are poorly defined.

The spatial structure of mature biofilms can be affected by the density of their founding populations. Specifically, experimental and theoretical evidence suggests that producer cells tend to segregate from non-producer cells when the cell density of the founding population is low, but not when it is high (van Gestel et al., 2014; Kreft, 2004). Producer cells are more likely to persist when they are spatially segregated from non-producer cells since spatial segregation reduces the chance of non-producer cells free-riding on producer cells (Nadell et al., 2010). The level of segregation depends on the cell density of the founding population possibly because cooperators cannot push away non-cooperators through cell division if there are too many cells. That is, when population density is sufficiently high, cell division is more likely to cause cell lineages to merge with each other (van Gestel et al., 2014; Persat et al., 2015). In this way, population bottlenecks could restrain free-riding in mature biofilms by giving cooperators the upper hand at the onset of biofilm formation.

A better understanding of how microbial aggregates respond to bottlenecks impacts key issues in evolutionary biology. This section focused on three issues: the role of disturbance events in the formation of social groups, the evolution of life-cycles, and the spatial structure of microbial colonies. Other issues include the role of feedback loops between ecological and evolutionary dynamics (Post and Palkovacs, 2009), and the high-variability of genes controlling social traits (Greig and Travisano, 2004). One of the take-home messages of this paper is that the effect of bottlenecks on social evolution is complex in that bottlenecks can favor producers under certain circumstances but not in others. The next section motivates the hypothesis that this might occur because of how bottlenecks alter the risk of being a producer in a microbial aggregate.

3 Social dynamics in a nonlinear world

One way of modeling collective benefit is to view it as increasing linearly with the number of producers (as in the N-person prisoner's dilemma). In this type of model, doubling the number of producers would generate twice as much collective benefit. However, the collective benefit generated by public good production in microbes is often a nonlinear function of the number of producers (Damore and Gore, 2012; Hauert et al., 2006). When the concentration of a public good is sufficiently high, further increasing its concentration may not produce as much

For further details on this issue, see De Monte and Rainey (2014); Ereshefsky and Pedroso (2015, 2013); Griesemer (2016).

benefit due to diminishing returns (Foster, 2004). Moreover, certain collective benefits, such as protection against predators, require the aggregation of a critical number of cells to manifest (Matz and Kjelleberg, 2005). Accordingly, it is not surprising that public good production in microbes is often regulated by quorum sensing mechanisms which allow cells to switch their behaviors on and off based on population density (Parsek and Greenberg, 2005).

Different authors have suggested that the type of non-linearity associated with a public good is key for understanding how population bottlenecks affect the social dynamics within a cell aggregate. For instance, when *P. fluorescens* biofilms were subjected to frequent bottlenecks, the frequency of cooperators decreased relative to intermediate disturbance frequency, and most free-riders started to inhabit the broth phase outside of the biofilm. It was suggested that this most likely occurred because building a biofilm is only cost-effective when there are enough cells to "anchor the biofilm in place" in the air-broth interface (Brockhurst et al., 2007). That is, frequent bottlenecks kept the population density below the threshold required for cooperation to be cost-effective. A related situation occurs with invertase production in yeast. Invertase producers are favored when producers are rare in the population (Greig and Travisano, 2004). When the number of producers is below a certain level, nonetheless, there is not enough invertase in the medium to efficiently digest the existing sucrose, which causes both producers and non-producers to grow more slowly (Gore et al., 2009). As a result, if the initial population density is sufficiently low, subjecting such a population to daily bottlenecks can drive it to extinction, even when every cell in the population is a producer (Sanchez and Gore, 2013).[5] In other words, invertase producers can be favored at low-density regimes, but the population needs to be above a certain size and contain enough invertase producers to be able to survive seasonal population bottlenecks.

Laboratory experiments thus suggest that the impact of population bottleneck on microbial aggregates is partly due to the nonlinear benefit conferred by public goods. Accordingly, it would be instructive to have a model showing how nonlinear benefits affect the frequency of producers after bottleneck events. The Stag Hunt game provides an intuitive model of how nonlinear benefits affect social evolution (Skyrms, 2004). According to the original story that this game is based on, each hunter can either hunt a stag or a hare. Stags provide a higher payoff than hares. But there is a catch: no one can successfully hunt a stag

[5] The population bottlenecks in this study were daily cycles of dilution with a 667× dilution factor.

alone. Consequently, if you decide to hunt a stag but someone else decides to hunt a hare, you end up with nothing. Alternatively, you might opt for hunting a hare, which is risk-free but yields a smaller payoff than a stag. In this game, hunting a stag can be viewed as a case of cooperation whereas hunting a hare as a case of defection. In the Stag Hunt game, if an individual is likely to interact with defectors, the best strategy is to defect. Thus, the chance of meeting another cooperator has to be higher than a certain threshold for cooperators to become fully established in the population.

The Stag Hunt game represents cases in which multiple individuals need to cooperate to produce a collective benefit. However, the original formulation of the Stag Hunt game is too stringent in that the presence of a single defector is sufficient to block the production of the collective benefit (Skyrms, 2004). However, public good production in microbes is often redundant in the sense that not every cell needs to be a producer to generate a group benefit. A bacterial biofilm can hold up even when some of its inhabitants are free-riders and do not contribute to its construction (Rainey and Rainey, 2003; Vlamakis et al., 2008). In yeast, about 99% of the digested sugars generated from invertase production dissipates away from the producer cells, which suggests that not every cell needs to produce invertase in order to support the population (Gore et al., 2009). In fact, under realistic conditions, the maximal collective benefit generated by invertase production occurred when only a portion of the population contained producers (MacLean et al., 2010).

A way of adjusting the Stag Hunt game to better handle public good production by microbes is to assume that only a proper subset of the interacting cells needs to cooperate in order to generate the collective benefit. This type of dynamics leads to the well-known Volunteer's Dilemma from game theory (Diekmann, 1985). A familiar example of this dilemma is the 'bystander effect.' In this example, a group of people witnesses an accident and, although a group benefit is produced if some people volunteer to help the victim (e.g., relief of conscience), helping the victim is costly which incentivizes everyone to free-ride and hope that others will do it.

The bystander effect is an example of the Volunteer's Dilemma in which the actors are humans endowed with sophisticated cognitive skills. Nevertheless, as Archetti (2009a) observes, the Volunteer's Dilemma is general enough to apply to public good production in microbes.[6] In particular, in a microbial aggregate,

[6]In the original formulation of the Volunteer's Dilemma by Diekmann (1985), only one individual was required to volunteer to produce the collective good. Archetti (2009a) gener-

not every cell needs to produce the public good to obtain the collective benefit (e.g., protection against antibiotics); however, if not enough cells produce the public good, all cells in the aggregate end up worse off (e.g., they become more susceptible to antibiotics). Similar to the bystander effect, it is better for a cell if other cells volunteer, but not if there are not enough volunteers to generate the group benefit.

Despite being a fairly abstract concept, the Volunteer's Dilemma provides a minimal model for explaining how population bottlenecks affect the level of cooperation within microbial aggregates.[7] When the size of an aggregate decreases because of a bottleneck, cooperators can be favored because not as many cells can afford to free-ride on other cells without causing the aggregate to break apart. However, if the reduction in the aggregate size is too extreme, cells have an incentive to free-ride because living in a multicellular aggregate is no longer beneficial since there are not enough cells to generate the public benefit. For instance, as suggested by Brockhurst et al. (2007), the frequency of producers peaked at intermediate disturbance probably because frequent bottlenecks kept the population density below the threshold required to produce the collective benefit, whereas infrequent bottlenecks allowed the population density to surpass such a threshold.[8] In this way, the Volunteer's Dilemma provides an intuitive model of how bottlenecks can favor cooperation under certain circumstances but not in others.[9]

alizes Diekmann's game to allow for cases in which more than one volunteer is necessary to generate the collective benefit.

[7]One might worry that that the Volunteer's Dilemma is too simplistic because the collective benefit is modeled as a step function. However, as Archetti and Scheuring (2011) show, using an "S"-shaped function instead of a step function makes little difference in the qualitative behavior of the game.

[8]In fact, this way of explaining the effect of population bottlenecks on *P. fluorescens* biofilms is akin to the mathematical model proposed in Brockhurst et al. (2007). As in the Volunteer's Dilemma, their formal model assumes that the number of producers have to reach a critical value before building a biofilm becomes worthwhile. For further details, see 'Supplemental Data' of Brockhurst et al. (2007). Similar remarks apply to the mathematical model used to describe the effect of bottlenecks on yeast populations (Sanchez and Gore, 2013, Text S1).

[9]One of the reviewers asked why this article does not use the Snowdrift game as opposed to the Volunteer's Dilemma game for modeling public good production in microbes. There are a couple of reasons. One of the limitations of the Snowdrift game is that this game is typically formulated as a two-player game. However, public good production in microbial aggregates involves the interaction of multiple cells and, for this reason, an n-player game such as the Volunteer's Dilemma is preferable. Furthermore, the dynamics of the Volunteer's Dilemma game better matches the experimental results of public good production in microbes than the Snowdrift game. For example, the Snowdrift game predicts that the lower the proportion of cooperators in the population, the higher the payoff for being a cooperator. In contrast, the Volunteer's Dilemma accommodates the fact that the benefit generated by public production, such as protection against predators, may require the presence of more than one cooperator

One of the main predictions of the Volunteer's Dilemma is that cooperators and free-riders can coexist stably in the population (Pacheco et al., 2009; Archetti, 2009b,a; Bach et al., 2006). This occurs because every cell pays a cost higher than that of volunteering if not enough cells volunteer. Without knowing what other cells will do, the best strategy for a cell is to volunteer with a certain probability (Archetti, 2009a). This prediction is consistent with the observation that biofilms and other microbial groups are often composed of both cooperators and free-riders (Nadell et al., 2009; Rendueles and Ghigo, 2012; Elias and Banin, 2012; Sanchez and Gore, 2013). Accordingly, the Volunteer's Dilemma describes a mechanism that could constrain the evolution of free-riders that does not appeal to genetic relatedness, which challenges the commonly held assumption that costly cooperation in microbes requires sufficiently high genetic relatedness to evolve (Archetti and Scheuring, 2012). The Volunteer's Dilemma thus illustrates an alternate evolutionary mechanism for the evolution of costly cooperation that could operate in tandem with gene selection (Archetti, 2009a).

Under the Volunteer's Dilemma, the incentive for volunteering depends on the threshold of volunteers required to produce the collective benefit. All other things being equal, the higher the threshold, the higher the incentive to volunteer. Experiments involving invertase production by yeast suggest that population bottlenecks can increase the threshold of volunteers required to support a group because the resilience of a group to bottleneck events depends on the proportion of cooperators in the group. Specifically, Sanchez and Gore (2013) observed that, when yeast populations were subjected to an extremely narrow bottleneck ($32,000\times$ dilution factor), pure populations of cooperators survived but not populations that contained a mix of cooperators and free-riders. This suggests that a group might need a higher proportion of producers in order to properly cope with severe bottlenecks. Accordingly, population bottlenecks might foster cooperation by increasing the threshold of volunteers required to generate the collective benefit.

In brief, laboratory experiments show that the effect of bottlenecks on microbial aggregates depends on the size of the aggregate and the frequency of producers. The Volunteer's Dilemma provides a minimal model to articulate how bottlenecks could affect public good production in microbial groups (Archetti, 2009a; Archetti and Scheuring, 2012). Cells in a collective would be better off to free-ride on the benefit produced by other cells but, if enough cells fail to vol-

to manifest.

unteer, the collective benefit is not produced and every cell ends up in a worse position. Nonetheless, it should be noted that the Volunteer's Dilemma leaves out some factors that are probably relevant for how bottlenecks affect microbial groups, such as the chance that producers will mutate into free-riders. Yet, the Volunteer's Dilemma provides theoretical support for the hypothesis that the non-linearity associated to a public good could be one of the factors that causes bottlenecks to favor cooperators under certain conditions.

4 Conclusion

The persistence of costly cooperation requires the presence of a mechanism that diminishes the advantages of free-riding. Some of these mechanisms assume that individuals have the ability to police and suppress free-riders (Sachs et al., 2004). For instance, free-riding can be disfavored if individuals can punish others that fail to reciprocate (Trivers, 1971), or when free-riders tarnish their reputation in the community (Nowak and Sigmund, 1998). With population bottlenecks, it is as if extrinsic ecological factors partly carry the burden of suppressing free-riders—as opposed to the members of a multicellular aggregate—by creating conditions in which free-riders are selected against. Accordingly, population bottlenecks illustrate how cooperators might persist in microbial collectives despite lacking certain cognitive skills, such as memory.

However, bottlenecks do not always foster cooperation. Laboratory experiments indicate that bottlenecks can also make free-riding more likely. Different authors have suggested that this is partly because the collective benefit generated by public good production, such as the sticky matrix in *P. fluorescens* biofilms, depends on the number of cells present (Brockhurst et al., 2007). The concept of the Volunteer's Dilemma provides one way of formalizing this argument. According to the Volunteer's Dilemma, when cells associate with a multicellular aggregate that is highly beneficial for them, the smaller the size of the aggregate, the higher the incentive for the cells to volunteer, *unless* the number of cells is below the threshold necessary to produce the collective benefit. In other words, population bottlenecks can foster cooperation or free-riding by altering the probability that the cells within an aggregate will volunteer.

Biofilms and other microbial communities are often a mix of cooperators and free-riders (Elias and Banin, 2012; Rendueles and Ghigo, 2012). This suggests that a more pertinent question to ask is not whether cooperating or free-riding

is the dominant strategy, but how cooperators and free-riders can coexist stably within microbial aggregates. The role of population bottlenecks in microbial social evolution suggests that the microbial lifestyle constantly alternates between selective regimes that favor cooperators and free-riders. For example, although population bottlenecks might initially favor producers, free-riders can eventually get the upper hand once the collective becomes large enough (Rainey and Rainey, 2003; Brockhurst, 2007). Accordingly, it is not surprising that gene expression in biofilms depends on population density (Parsek and Greenberg, 2005), that invertase expression in yeast is repressed at higher concentrations of glucose (Gore et al., 2009), and that genes coding for social traits are highly polymorphic (Greig and Travisano, 2004). In agreement with these experiments, the Volunteer's Dilemma offers a way of understanding social conflicts in microbial aggregates in which neither cooperating nor free-riding is the dominant strategy.

Conflict of interest

The author has no conflict of interest to declare.

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