

REVIEW

The ecology and evolution of social behavior in microbes

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ABSTRACT

Cooperation has been studied extensively across the tree of life, from eusociality in insects to social behavior in humans, but it is only recently that a social dimension has been recognized and extensively explored for microbes. Research into microbial cooperation has accelerated dramatically and microbes have become a favorite system because of their fast evolution, their convenience as lab study systems and the opportunity for molecular investigations. However, the study of microbes also poses significant challenges, such as a lack of knowledge and an inaccessibility of the ecological context (used here to include both the abiotic and the biotic environment) under which the trait deemed cooperative has evolved and is maintained. I review the experimental and theoretical evidence in support of the limitations of the study of social behavior in microbes in the absence of an ecological context. I discuss both the need and the opportunities for experimental investigations that can inform a theoretical framework able to reframe the general questions of social behavior in a clear ecological context and to account for eco-evolutionary feedback.

KEY WORDS: Cooperation, Eco-evolutionary dynamics, Sociobiology

Introduction

The study of cooperation has preoccupied biologists for centuries but the potential for social behavior in microbes has only recently been recognized. Established examples include collective hunting by *Myxococcus* (Velicer and Vos, 2009), aggregation and subsequent cell death in stalks of *Dictyostelium* (Bonner, 2009; Strassmann and Queller, 2011) and biofilm formation, such as in the mats of *Pseudomonas fluorescens* (Rainey and Rainey, 2003) or *Bacillus subtilis* (van Gestel et al., 2014). Since the recognition of this social dimension, research into microbial cooperation has accelerated and microbes have become an ideal model system because of their fast evolution, their convenience for lab use and the opportunity for molecular and genetic investigations.

However, the study of microbes also poses significant challenges, foremost among which is a lack of knowledge of the ecological context under which the supposedly cooperative trait has evolved and is maintained (O'Brien and Brockhurst, 2015; Rainey, 2015; Zhang and Rainey, 2013). Here, ecology encapsulates both the biotic (interactions among organisms) and the abiotic (interactions between organisms and their environment) dimensions (see Glossary). When seemingly cooperative and cheating traits have been studied in different ecological contexts, a variety of genotype-by-environment interactions have been found that have questioned the appropriateness of sociobiological labels (Driscoll et al., 2011;

Dubravcic et al., 2014; Rainey et al., 2014; Redfield, 2002; Tarnita et al., 2015; Zhang and Rainey, 2013). Moreover, the rapid evolutionary pace also entails that traits can be selected for on a fast timescale that affects the ecology of the microbes, from intraspecific and interspecific interactions to the level of the ecosystem (Lennon and Deneff, 2015), leading to crucial eco-evolutionary feedback. Consequently, the casting of most intraspecific (and more recently also interspecific) interactions and extracellular excretions under the cooperation/conflict umbrella in the absence of an ecological context can lead to misleading or incomplete descriptions of the behavior (Driscoll et al., 2011; Dubravcic et al., 2014; Rainey et al., 2014; Redfield, 2002; Tarnita et al., 2015; Wolf et al., 2015; Zhang and Rainey, 2013).

A revision of the cooperation framework to account for ecology and eco-evolutionary feedbacks seems in order; however, forays into microbial ecology have proved remarkably challenging in general, and this has stunted the advance of many disciplines from the study of gut microbiota with its relevance for human health, to that of soil or coral reef microbiota with their relevance for biodiversity conservation (de Vrieze, 2015; Knowlton and Rohwer, 2003), to that of antibiotic development and its relevance for the looming health threat of antibiotic resistance (Kaeberlein et al., 2002). Not only do we not have a grasp of microbial intraspecific and interspecific interactions but also the convenience of lab investigations is illusory as in fact the majority of microbes have not yet been successfully cultured, most likely because of the absence of their ecological context (Kaeberlein et al., 2002). However, recent progress has been made in this and other respects, giving hope for the study of microbial ecology in general, and therefore also for the study of social behavior in the appropriate ecological context.

Here, I review the experimental and theoretical evidence in support of the limitations of the study of social behavior in microbes in the absence of an ecological context. I discuss both the need and the opportunities for experimental investigations and for building a unifying framework that reframes the general questions of social behavior in terms of ecology and eco-evolutionary feedback. Such a framework would inform not only the study of social behavior in microbes but also, more generally, the study of microbial communities.

The cooperative dilemma and mechanisms for the evolution of cooperation

Definitions

Varied definitions can be found in the literature on cooperation. A necessary condition pertaining to all definitions is that a cooperative act must have positive consequences for the fitness of the recipients. To some, this is also a sufficient condition (West et al., 2006); if, moreover, the consequences to the cooperator are also positive, then the behavior is called mutually beneficial; otherwise, it is called altruistic. To others, only the latter scenario qualifies as cooperation (Nowak, 2006). Furthermore, the term cooperation is typically reserved for intraspecific interactions, though there have been recent efforts to extend it to interspecific interactions as well (e.g. Coyte

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Glossary**Cooperation**

Costly behavior that confers fitness benefits on same-species recipients.

Defection (free-riding)

Absence of the cooperative behavior coupled with exploitation of its benefits.

Ecology

Interactions among organisms and between organisms and their environment.

Public good

A costly resource produced by the cooperator and freely available to others.

Semi-public good

A good that is partially public and partially private.

et al., 2015). Because, as I will argue below, a cooperative label is challenging and can be misleading to apply to microbes even when focusing only on intraspecific interactions, here I will focus exclusively on intraspecific interactions and will define cooperation to be a costly behavior that confers fitness benefits on same-species recipients (see Glossary). With this definition, the dilemma of cooperation can be summarized as follows: why would an individual give up part of its own fitness to confer fitness benefits on other individuals?

One has to exercise care, however, in assessing whether a cooperative dilemma actually exists: for example, a behavior may appear to be costly but if it actually provides large enough long-term benefits that it entails very small to non-existent lifetime fitness costs (e.g. group hunting in African wild dogs, territorial choruses in lions, polyandry in Galapagos hawks, pleometrotic ants, or breeding associations in bark beetles) then there is no dilemma (Avilés et al., 2004; Bernasconi and Strassmann, 1999; Clutton-Brock, 2009; Kokko et al., 2001).

Cooperation and public good

Cooperation has been conceptualized in two ways: either as a costly donation in a pairwise interaction between a donor and a recipient (described via a Prisoner's Dilemma game; Nowak, 2006) or as a public good, a costly resource produced by the cooperator and freely available to others (see Glossary). For microbes, typically the latter conceptualization has been deemed more appropriate (West et al., 2006). It is essential to stress here that there are multiple definitions of what constitutes a public good in the literature. Economists define a public good to be both non-excludable (freely available) and non-rivalrous (use of the good by one individual does not reduce the amount available to other individuals, e.g. defense or education). Because in biology the produced good is typically a finite resource, a biological public good is a good that is non-excludable (freely available) but not necessarily non-rivalrous. There are some who argue that a public good should be defined more broadly to include any good that is not entirely excludable/private (Kümmerli and Ross-Gillespie, 2013). However, this can lead to confusion as a public good is typically associated with a cooperative dilemma whereas a partially private good may not necessarily pose the same dilemma if the direct benefits to the producers outweigh the costs of production. In fact, finding ways to privatize public goods is one mechanism for avoiding the cooperative dilemma (Allen et al., 2013; Drescher et al., 2014; Gore et al., 2009; Rainey et al., 2014). In this review, I make the useful distinction between a public good that is freely available and a semi-public good that is partially private (see Glossary).

Regardless of whether we look at a Prisoner's Dilemma or at a public goods game, both in theoretical models and in experiments, non-cooperative individuals (also called defectors, free-riders or non-producers; see Glossary) derive benefits from their interactions with cooperators without paying any cooperative cost. In a well-mixed population in which individuals interact with equal probability with others or have equal access to the public good, defectors always have a higher fitness than cooperators, and natural selection acts to increase their number until cooperating types go extinct (Hofbauer and Sigmund, 2003; Nowak, 2006) – a phenomenon known as the tragedy of the commons (Hardin, 1968). This outcome can be avoided in several ways. First, if the cooperative behavior appreciably increases the carrying capacity of the population, the larger population size achieved by cooperators is less sensitive to demographic noise and is therefore less likely to be invaded by defectors (Constable et al., 2016; Houchmandzadeh and Vallade, 2012). Although demographic stochasticity is most effective at small population sizes that are less relevant for microbes, as long as the large microbe populations are spatially structured into smaller meta-populations, the same mechanism above will promote cooperation (Constable et al., 2016). Second, the cooperative behavior may be able to persist in spite of exploitation by cheaters if certain phenotypes exist that cannot be exploited by cheaters but which allow the successful regeneration of the cooperative behavior. An example is a phenotype that neither uses the public good nor produces it (often referred to as a loner, a generic term that reflects the lack of participation in the social dynamics of interest): when the non-producers proliferate and all but eliminate the producers, the public good declines and the non-producers eventually derive lower and lower payoffs; the loners then have a higher payoff, proliferate and drive down the number of non-producers, allowing producers to thrive again. This creates a cyclical dynamic, reminiscent of rock–paper–scissors, that allows for the persistence of cooperators (Hauert et al., 2002; Inglis et al., 2016). Third, in the absence of such mechanisms, the evolution of cooperation requires some form of assortment that makes cooperators more likely to interact with other cooperators on average (Fletcher and Doebeli, 2010; Nowak, 2006). In that context, individual-level costs can be outweighed by the social benefits derived from interacting with other cooperators.

Mechanisms of assortment

Multiple mechanisms have been proposed to achieve assortment (see Nowak, 2006, for a review), such as: (i) the existence of an underlying spatial structure combined with limited dispersal, also known as population viscosity, which clusters together parents and offspring (Hamilton, 1964; Lieberman et al., 2005; Nowak and May, 1992; Nowak et al., 2010a; Pfeiffer and Bonhoeffer, 2003; Tamita et al., 2009b; West et al., 2006); (ii) structure arising from the properties of the individuals such as adhesion, speed of movement or perception of environmental cues (Avilés, 2002; Garcia et al., 2014, 2015); (iii) recognition of like or discrimination of non-like based on various cues such as kinship, phenotypic similarity, signaling or shared preferences (Antal et al., 2009; Mehdiabadi et al., 2006; Ostrowski et al., 2008; Pacheco et al., 2006; Rousset and Roze, 2007; Stefanic et al., 2015; Tarnita et al., 2009a; Traulsen and Nowak, 2007; Vos and Velicer, 2009); (iv) reciprocity, which relies on either memory or reputation to ensure that future interactions are informed by previous ones (Axelrod and Hamilton, 1981; Nowak and Sigmund, 1989, 2005; Trivers, 1971); or (v) multilevel selection (Traulsen and Nowak, 2006; Wilson, 1975), which considers selective pressures both at the individual

and at the group level and favors cooperator-dominated groups. When the mechanisms above lead to assortment between genetically related individuals, kin selection – a process by which traits change in frequency over evolutionary time in part because of their effects on the fitness of genetically related individuals – is said to occur (West et al., 2006).

While such mechanisms can promote the evolution of cooperation, they are not sufficient: the benefits of cooperation derived under the various types of assortment have to outweigh the costs of the cooperative behavior as well as the costs of intensified local competition, which is an unavoidable consequence of assortment. Theoretical examples abound where despite successful assortment a cooperative behavior can never evolve, regardless of the magnitude of its benefit or cost (Grafen, 2007; Ohtsuki and Nowak, 2006; Ohtsuki et al., 2006; Taylor et al., 2007).

Finally, it is important to note that cooperation does not imply the impossibility or the absence of conflict (Rainey and De Monte, 2014): highly social groups are characterized by strong mechanisms that enforce cooperation such as policing in ant colonies, ostracism/punishment in animal groups, or the presence of an immune system in complex multicellular organisms. And yet conflict can arise such as in the case of cancer in multicellular organisms: cancerous cells are cells that ignore the developmental program and reproduce uncontrollably to the detriment of the group (Tarnita et al., 2013).

Limitations of the theory of cooperation and implications for microbial behavior

Theory is supposed to simplify in order to capture the essence of a phenomenon. When it comes to cooperation, rich and detailed investigations of assortment mechanisms that have resulted in a multitude of insights have come at the cost of a simplified description of ecology, which is typically subsumed into the benefits and costs of the cooperative behavior (Nowak, 2006). For example, it is often the case that for social interactions to occur, first a group must form (van Veelen et al., 2010). Two elementary operations – called alternatively unitary, non-divisional or staying together (ST) versus aggregative or coming together (CT) – have been implicated in all known events of group construction (Bonner, 1998; Grosberg and Strathmann, 2007; Queller, 2000; Tarnita et al., 2013). In ST, individuals form groups by not separating (or dispersing) after reproduction. In CT, independent individuals (possibly of different genotypes/species) form aggregates. Because of the focus on assortment, the two mechanisms of group formation (ST versus CT) have been mostly compared based on their assortment outcome rather than in the ecological context in which they emerged, despite the fact that existing evidence points to ecology as the main determinant of the type of emerging group formation and social organization (Avilés et al., 2004; Bonner, 1998; Jarman, 1974; Kocher et al., 2014; Ross and Keller, 1995). This obscures an important connection between the type of emerging cooperation (and whether the behavior is cooperative at all) and the ecological role it is selected to fulfill. It is not possible to determine whether and how successfully the problem of cooperation has been solved by a group without understanding what a successful resolution entails for that group. For example, success in a given ecological context may require a rapid but transient cooperative grouping of a large number of individuals, while in a different context it may require long-term maintenance of cooperation. This does not make the former a less-successful cooperation than the latter.

When cooperation has been studied in specific cases, a suite of complex ecological factors has been shown to play an essential role

in the evolution of cooperation. For example, spatiotemporal environmental variation (Rubenstein, 2011) and lack of available breeding habitat (Emlen and Wrege, 1989) are important drivers for cooperative breeding in birds; the dispersion and availability of food and its typical pattern of predator-avoidance behavior are critical factors determining the group size and social organization of African antelope species (Jarman, 1974); strong intraspecific competition can lead to associations, albeit short lived, between unrelated ant queens (Bernasconi and Strassmann, 1999; Ross and Keller, 1995); and altitudinal gradients with their impacts on the length of the breeding season seem to be key determinants for whether various species of hymenoptera develop a highly social, intermediately social or a solitary lifestyle (Kocher et al., 2014). For such cases, the theory of cooperation has provided useful general insights but a complete understanding of their cooperative behaviors has required in-depth knowledge of ecological factors that the theory of cooperation in its general form misses. While this limitation can be remedied to a certain extent in organisms whose ecology is accessible, it represents a serious challenge for the study of microbes with their largely inaccessible ecology. In the following three subsections, I first review what sociobiology has taught us about microbes and, subsequently, I review the evidence for a need to expand theoretical and experimental investigations to *in vivo* conditions that include ecology and eco-evolutionary feedback.

General framework and findings

Based on existing theoretical insights, the main approach to the sociobiological study of microbes has been to examine the consequences of the supposedly social trait in a social context and determine the costs and benefits to the donor and the recipients. Typically, the main step is to determine the relative fitness of the wild-type (which possesses the supposedly social trait) and the mutant (which does not possess the supposedly social trait but is able to reap its benefits), both in monoculture and in mixed culture. A subsequent step is to do this both under conditions in which the behavior is needed and under conditions in which it is not (West et al., 2006). For example, if the behavior under study is costly invertase production, which can break down sucrose into fructose and glucose, the latter of which can then be taken up by cells, then the benefit of the behavior can be altered by varying the amount of glucose already available in the media (Gore et al., 2009). One of the advantages of working with microbes is that the investigations above are facilitated by the ease with which mutants that do not perform the behavior can be obtained [either by artificially knocking out the gene(s) responsible for the cooperative behavior or by spontaneous mutation] (West et al., 2006).

The predictions of sociobiology theory are that in media where the behavior is needed: (i) cooperators will do better than cheaters in monoculture; (ii) cooperators will do worse than cheaters in mixed culture; and (iii) in mixes, cheaters will do best when they are rare and in denser populations. If the behavior is not needed, then cooperators have no advantage, even in monoculture. A further set of experiments tests for conflicts between non-relatives (e.g. when multiple strains aggregate to form a fruiting body such as in *Dictyostelium discoideum* or *Myxococcus xanthus*) and predicts that the more distantly related the strains, the greater the conflict between them. This is expected to translate into one strain exploiting the other (reproductive skew) and possible overall reduced success of the fruiting body (Strassmann and Queller, 2011; Velicer and Vos, 2009; West et al., 2006). Below, I discuss specific examples that show how these reasonable theoretical predictions can lead to misleading results and incorrect/incomplete

interpretations when applied in the absence of an ecological context.

Public good production: not all extracellular products are public goods; not all non-producers are social cheaters

Pyoverdinin – an iron-chelating molecule produced by bacteria from the genus *Pseudomonas* and involved in the uptake of ferric iron – is one of the best-studied extracellular products (see Zhang and Rainey, 2013, and references therein). Pyoverdinin is hypothesized to be a public good: lab experiments confirmed sociobiological predictions that producers outperform non-producers in monoculture but are outperformed by non-producers in mixed culture (Griffin et al., 2004). However, Zhang and Rainey (2013) hypothesized that uncertainties about the ecophysiology of pyoverdinin make critical the choice of experimental conditions in which the sociobiology of pyoverdinin is investigated. They set up experiments to follow the evolution of producers and subsequent emergence of non-producers in an environment rich in bio-unavailable iron (i.e. an environment that favors pyoverdinin production). They then proceeded to test the sociobiological predictions by performing growth and fitness assays of producers and non-producers in the same environment in which they evolved. They found that agreement with the sociobiological predictions depended strongly on both genotype and environment. Whereas in some environments non-producers indeed behave like cheaters, in others they evolve because pyoverdinin production is maladaptive; whereas under certain conditions producers can be exploited by cheaters, under others they are able to privatize pyoverdinin, making it a semi-public rather than a public good.

These findings highlight that the ecology, ecophysiology, genetics and cell biology of pyoverdinin are far richer than appreciated in the context of sociobiological experiments (Rainey et al., 2014; Zhang and Rainey, 2013; but see also Julou et al., 2013) and draw attention to the artificial nature of lab environments and the need for studies in natural settings. The authors conclude by suggesting several alternative hypotheses that account for the ecological significance of producers and non-producers (Zhang and Rainey, 2013).

Conflicts between non-relatives: not all social asymmetry implies cheating and social conflict

Upon starvation, solitary *D. discoideum* amoebae aggregate with neighbors to form a multicellular fruiting body made of a stalk and spores. The spores are resistant to starvation and will germinate upon encountering favorable conditions, while the stalk cells die during stalk development (Bonner, 1982, 2009; Raper, 1984; Strassmann and Queller, 2011). In the process of aggregation, these amoebae do not perfectly discriminate against non-kin; consequently, chimeras (i.e. multicellular fruiting bodies consisting of ≥ 2 genotypes) have been observed both in the lab and in nature (Fortunato et al., 2003b; Gilbert et al., 2007; Sathe et al., 2010; Strassmann et al., 2000). These chimeras are functional: the multiple genotypes participate in both stalk formation and spore production (although not necessarily in equal measures, a phenomenon known as reproductive skew; Strassmann et al., 2000). Therefore, this is an ideal organism for the study of potentially very costly social behavior (Strassmann and Queller, 2011). Studies to date have found significant reproductive skew in *D. discoideum* chimeras (Buttery et al., 2009; Fortunato et al., 2003a) and in a variety of other cellular slime molds (Sathe et al., 2014). Because some strains are always dominant (over-represented in the spores of chimeras) and others are always

subordinate (under-represented in the spores of chimeras) (Buttery et al., 2009; Fortunato et al., 2003a; Sathe et al., 2014), in the absence of additional frequency-dependent processes that maintain coexistence, the subordinate strains should eventually be outcompeted in the wild. Therefore, these findings point towards a decrease in species-wide genetic diversity that is inconsistent with the immense diversity and coexistence found among wild strains (Fortunato et al., 2003a,b; Sathe et al., 2014, 2010).

Recent studies (Tarnita et al., 2015; Wolf et al., 2015) have suggested that this inconsistency arises as a result of the one-dimensional assessment of *D. discoideum* fitness, which is equated solely with spore contribution, and proposed that life-history trade-offs between non-social traits lead to multiple fitness components. One of these is the empirically determined trade-off between spore number and viability: genotypes that were over-represented in spores also made smaller and less-viable spores when grown clonally (Wolf et al., 2015). A second proposed trade-off is between staying vegetative and becoming a spore (Dubravcic et al., 2014; Tarnita et al., 2015). In *D. discoideum*, not all cells aggregate to become multicellular; these non-aggregating, ‘loner’ cells are viable: upon food replenishment, they eat, divide and produce progeny that can aggregate in response to starvation. Theoretical studies suggested that the loners might reflect a bet-hedging strategy in uncertain environments: loners have a high chance of death if the starvation period is long, but, if food does return to the environment before they die, they will benefit from priority effects (i.e. a head start) relative to spores that need time to germinate. Tarnita et al. (2015) hypothesized that genotypes over-represented in spores might simply be those that have been selected to leave fewer loners behind and thus, that the social cheating is only apparent. Given spatial or temporal environmental heterogeneity, this differential investment in loners versus spores is sufficient to produce a large diversity of coexisting genotypes and resolve the coexistence inconsistency (Martinez-Garcia and Tarnita, 2016a preprint; Tarnita et al., 2015).

Extending the same well-mixed model that assumes no social interactions to include multiple life-history traits and trade-offs between non-social traits qualitatively recapitulates all existing empirical results that have been attributed to social behavior (Martinez-Garcia and Tarnita, 2016b). While this finding does not suggest that social interactions do not exist, it shows that they are not necessary to qualitatively explain the observed behaviors and emphasizes that additional quantitative investigations are needed to determine whether any type of social interactions do exist in *D. discoideum*.

Most importantly, these findings showcase the complex outcomes that selection on non-social traits in complex ecological contexts can produce in the absence of any social interactions, and highlight the misleading interpretations that can arise from sociobiological investigations into microbial behavior that are not grounded in ecology.

The dangers of anthropomorphizing: when does cooperation cease to be a useful concept?

In addition to the ecological limitations to the theory of cooperation, there is also a non-trivial challenge intrinsic in the semantics of the field. One problem with casting the world in the light of a cooperative framework is that it necessarily implies that bad guys (cheaters) arise and erode at the cooperative foundation. This, as explained above, leads to a dead-end, tragedy of the commons conclusion, unless mechanisms are in place to help cooperation

persist. This focus on persistence, however, while so far very informative, comes at the expense of: (i) alternative hypotheses that might reveal different roles and different types of interactions between the purported cooperators and cheaters; and (ii) the possibility that cooperation is not always an end in itself but that both cooperation and cheating could form a transient phase serving as a stepping stone for new interactions and behaviors. These points are elegantly emphasized by studies in two bacteria: *P. fluorescens* (Hammerschmidt et al., 2014; Rainey and Kerr, 2010; Rainey and Rainey, 2003; Rainey and Travisano, 1998) and *M. xanthus* (Fiegna et al., 2006; Velicer and Vos, 2009).

Cheaters as essential propagules: *Pseudomonas fluorescens*

In a structured environment (a beaker filled with broth), populations of a freely swimming *P. fluorescens* genotype (called ‘smooth’ or SM) quickly and spontaneously mutate to produce ‘wrinkly spreader’ (WS) cells that swim to the air–broth interface, produce a glue that prevents daughter cells from separating after division and consequently form a mat. Although glue production is costly to individual WS cells, they benefit from the access to oxygen and the trait spreads. Soon after the mat forms, however, selection on individual cells leads to the emergence of cheating SM types: cells that do not produce the glue but are nevertheless able to reap the benefits of mat formation because they accumulate on top of the mat, where they have access to oxygen. Eventually, the mat collapses under the weight of the cheating pile and all WS cells die. However, SM cells have the potential to reintroduce the WS type via spontaneous mutation. This inspired Hammerschmidt et al. (2014) to consider an ecological scenario in which cheating SM cells are integral to the persistence of the mat phenotype. They set up a large-scale experiment consisting of hundreds of microcosms: WS cells initiate each microcosm; cheater SM cells soon follow; the mat collapses; and, in a few of the microcosms, SM cells mutate back into WS type. This constitutes a successful microcosm: one that is able to preserve the cyclical dynamics of WS and SM types. Whenever a microcosm fails (i.e. the cycle is broken), it gets reseeded with cells from a successful microcosm. Ecologically, this encapsulates a scenario in which persisting cyclical behavior on some liquid surface (e.g. a lake) allows for dispersal of SM types to colonize empty habitat. Although a rare mutation process is required in the beginning to maintain the cycle (leading to lots of failed microcosms), eventually an integrated life cycle emerges that alternates between phenotypic states without the need for mutations. SM and WS emerge as two phenotypes produced by the same genotype: WS ensures successful access to oxygen but a mat that keeps growing will eventually collapse under its own weight, even in the absence of cheaters; the persistence and dispersal of the WS phenotype is then ensured by the apparent SM cheaters, which play the role of propagules (Rainey and Rainey, 2003).

This shows that under certain ecological conditions, apparent cheaters could in fact be an integral part of the survival strategy of the producers. Such observations are not restricted to microbes. Defectors as propagules are also reminiscent of eusociality, a type of social behavior that is characterized by overlapping generations and division of reproduction (certain individuals reproduce while others do not) (Nowak et al., 2010b). Eusocial ant colonies consist of a queen (the sole reproducer), her sterile worker daughters (possibly divided into multiple castes performing different tasks) that help raise the offspring of the queen, and her virgin queen daughters that do not help at the nest but fly out at maturity, mate and start their own colonies. Typically, the worker ant that stays at the nest and helps raise more sisters is considered to be altruistic and explaining its

behavior has led to decades of scientific investigations. However, if the worker ant is indeed an epitome of cooperation, then the virgin queen that does not help, but flies away to start her own colony, could be seen as a selfish individual. This of course is not the case, as both types are needed for the success of eusociality (Nowak et al., 2010b). The virgin queen is simply the propagule that continues the life cycle, even after the death of the mother colony, and spreads the eusocial behavior. Although the parallels are clear, for eusocial colonies this idea is not as surprising as the results of Hammerschmidt et al. (2014) for microbes, arguably because for eusociality we have a better understanding of ecology and development, which places cooperation more firmly in the appropriate context. In the absence of such a context for the study of microbes, it seems paramount to carefully consider the terms we employ to describe behavior and interactions; the use of terms such as cooperation, cheating and selfishness poses the danger of anthropomorphizing behaviors and of biasing our study of evolutionary processes. More neutral terms such as producers and non-producers are preferable, as they allow for multiple alternative hypotheses to be considered (Rainey et al., 2014), leading to an exploration of different ecological scenarios.

Cheaters as an evolutionary stepping-stone: *Myxococcus xanthus*

Typically, models of cooperation consider only the two traits – cooperation versus cheating – without the possibility of innovation. However, in nature, especially in the case of microbes and their evolution on fast time scales, both cooperation and cheating might play crucial roles as part of a transient phase that can allow for subsequent innovation. An interesting example comes from *M. xanthus*. These bacteria collectively prey on a broad range of microorganisms; upon starvation, they undergo a development that terminates with the differentiation of rod-shaped cells into spherical spores within a multicellular fruiting body. However, not all cells become spores: some undergo autolytic death (though whether they provide any benefit to the spores by doing so is unclear), while others remain as loner rod-shaped cells that circle the fruiting body perimeter (Velicer and Vos, 2009). The death of a significant fraction of the aggregated cells implies that fruiting body formation is a costly process and allows for the possibility of cheaters. One such cheater fails to produce viable spores in monoculture, which makes it an obligate social cheater whose survival during starvation is dependent upon chimeric fruiting body development with a social host. In lab experiments, this obligate cheater, which led to the downfall of cooperation, eventually mutated into a novel social type; moreover, it did so via mutations that generated novel genetic interactions rather than by a simple reversal of its defects. Thus, ‘a temporary state of obligate cheating served as an evolutionary stepping-stone to a novel state of autonomous social dominance’ (Fiegna et al., 2006).

Future directions

As the role of microbes as major players in ecosystems is increasingly recognized, the inaccessibility of their ecology appears to be ever more critical. In agriculture, where microbes are hailed as the best hope for the recovery of degraded soils (de Vrieze, 2015), traditional investigations that started with extensive lab and greenhouse studies to determine the most promising strains eventually proved unsuccessful, as those strains were ineffective in the field ‘because of soil, climate, and ecosystem effects’ (de Vrieze, 2015). Now a ‘field-first approach’ is employed in which combinations (sometimes hundreds or thousands) of strains are tested in field plots first, and subsequently the most successful ones

are brought to the lab to try to determine their mechanisms of action. However, once back at the lab, the next limitation comes from an inability to culture most microbes, again, likely due to the absence of their ecological context. Recently, remarkable progress has been made in this area. Using innovative techniques, Kaerberlein et al. (2002) and Nichols et al. (2010) were able to grow uncultured organisms by cultivation in their natural environment: 50% of cells isolated from soil are reported to grow with this new technique, compared with 1% that are able to grow on a Petri dish. This approach has already yielded important results: extracts from 10,000 isolates grown with the new technique were screened for antimicrobial activity and a new class of antibiotics was discovered (Ling et al., 2015). This gives hope that behaviors of interest could now be studied in their own ecology, in addition to lab investigations.

An approach that is intermediate between the Petri dish and the natural environment has been to replicate some of the ecological context in the lab. This has yielded exciting results, with *B. subtilis* biofilm formation and properties (Bais, 2004) being studied in the soil, on plant roots in the lab, naturally induced by the plants' polysaccharides (Beauregard et al., 2013). Similarly, both synthetic communities constructed from known microbial species (Hekstra and Leibler, 2012; van Gestel et al., 2014) and microcosm communities made from environmental samples (Wolfe and Dutton, 2015) have been shown to reveal essential characteristics of microbial ecology (for a review, see Widder et al., 2016).

In parallel with such advances that will provide an ecological dimension to the prolific evolutionary, molecular, genomic and developmental studies of microbes, there have been exciting molecular and genomic advances in organisms whose ecology and sociality are much more accessible and better understood. For example, in social insects we are now able to study social genes and the way they interact with the social environment (Manfredini et al., 2013; Wang et al., 2014), to look at epigenetic signatures (Libbrecht et al., 2016), and to aim for an understanding of the molecular basis of social behavior.

Such advances position us at an ideal juncture where empirical insights are increasingly possible and likely to inform a rethinking of the theoretical framework of cooperation and social behavior in the broader context of interactions, development and ecology.

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