The Social Lives of Microbes

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Abstract

Our understanding of the social lives of microbes has been revolutionized over the past 20 years. It used to be assumed that bacteria and other microorganisms lived relatively independent unicellular lives, without the cooperative behaviors that have provoked so much interest in mammals, birds, and insects. However, a rapidly expanding body of research has completely overturned this idea, showing that microbes indulge in a variety of social behaviors involving complex systems of cooperation, communication, and synchronization. Work in this area has already provided some elegant experimental tests of social evolutionary theory, demonstrating the importance of factors such as relatedness, kin discrimination, competition between relatives, and enforcement of cooperation. Our aim here is to review these social behaviors, emphasizing the unique opportunities they offer for testing existing evolutionary theory as well as highlighting the novel theoretical problems that they pose.

1. INTRODUCTION

Microorganisms exhibit a stunning array of social behaviors (Crespi 2001). Individuals communicate and cooperate to perform activities such as dispersal, foraging, construction of biofilms, reproduction, chemical warfare, and signaling. Microbiologists are making amazing advances in our understanding of these behaviors from a mechanistic perspective, examining the molecular mechanisms involved and the underlying genetic regulation (Kolter & Greenberg 2006, Lazdunski et al. 2004, Parsek & Greenberg 2005, Webb et al. 2003, Williams et al. 2007).

Our aim in this review is to highlight the numerous opportunities microbes offer for evolutionary biologists and ecologists interested in social evolution. First, there is a huge amount of microbe biology requiring an evolutionary explanation. Cooperation and communication appear to be extremely important to microbes; for example, 6–10% of all genes in the bacterium *Pseudomonas aeruginosa* are controlled by cell-cell signaling systems (Schuster et al. 2003). Consequently, if explaining cooperation is one of the greatest problems for evolutionary biology, then explaining cooperation in microbes is one of the key aspects of this problem. Second, social evolution theory was largely developed to explain known behaviors in animals such as insects, mammals, and birds. The huge variety of social behaviors discovered in microbes offers a unique opportunity to test how generally that theory can be applied to other taxa, which did not play a major role in the development of that theory. Furthermore, microbes provide most of the diversity of life, and so previous focus on animals may have given a misleading impression of the importance of different factors for social evolution.

Third, microbe systems are uniquely amenable to experimental study: They have short generation times for selection experiments, and genetic mutants that do not cooperate (cheats) can be created relatively easily. Fourth, the biology of microbes means they offer some novel problems. For example, genes for social behaviors can be transferred horizontally between different bacterial lineages, by mobile genetic elements (Smith 2001). Fifth, the social lives of microbes have a significant impact on human life; cooperative behaviors play key roles in the ability of microbes to (*a*) infect and harm humans, livestock, and agricultural plants (André & Godelle 2005, Williams et al. 2000) and (*b*) provide beneficial services such as breaking down sewage (Valle et al. 2004) or symbiotically aiding plant growth (Kiers et al. 2002).

In the first part of this review, we provide a brief summary of social evolution theory relevant to microbes (Section 2). In the main parts of the paper, we give a tour of microbial social behaviors (Sections 3 and 4). To highlight the general issues, we discuss two categories of social behavior in some detail: the production of public goods and fruiting bodies (Section 3). In the final sections, we consider two topics that arise repeatedly: the implications for the evolution of parasite virulence (Section 5) and the application of the kin selection coefficient of relatedness to microorganisms (Section 6). Although this paper is aimed primarily at evolutionary biologists and ecologists, interdisciplinary research is key to this field, with microbiologists discovering behaviors that need evolutionary explanations and the evolutionary and ecological

approaches providing problems for which there must be a mechanistic answer (West et al. 2006).

2. SOCIAL EVOLUTION THEORY

Theoretical explanations for any behavior can be broadly classified into two categories: direct and indirect fitness benefits (Grafen 1984, Lehmann & Keller 2006, Sachs et al. 2004, West et al. 2006). This follows from Hamilton's (1964) insight that individuals gain inclusive fitness through their impact on the reproduction of related individuals (indirect fitness effects) as well as directly through their impact on their own reproduction (direct fitness effects). Cooperative behaviors that benefit other individuals have posed particular problems for evolutionary biologists because although the cost of a cooperative behavior may be very obvious, the benefits are often obscure. We do not discuss the use and misuse of terms such as cooperation and altruism, as we have recently covered that in detail elsewhere (West et al. 2007).

The first explanation for the evolution of cooperation is that it provides a direct fitness benefit to the individual that performs the behavior, which outweighs the cost of performing the behavior. In this case, cooperation is mutually beneficial (West et al. 2007). One possibility is that individuals have a shared interest in cooperation. For example, in many cooperative breeding species, larger group size may benefit all the members of the group through factors such as greater survival or higher foraging success. In this case, individuals can be selected to help rear offspring that are not their own to increase group size (Kokko et al. 2001). Another possibility is that there is some mechanism for enforcing cooperation, by rewarding cooperators or punishing cheaters (Frank 2003, Trivers 1971). This could happen in a variety of ways, which have been termed punishment, policing, sanctions, reciprocal altruism, indirect (reputation-based) reciprocity, and strong reciprocity.

The second class of explanations for cooperation is that it provides an indirect benefit by improving the fitness of other individuals who carry the cooperative gene (Hamilton 1964). In this case, cooperation is altruistic. The simplest and most common reason for two individuals to share genes in common is for them to be genealogical relatives (kin), and so this is often termed kin selection (Maynard Smith 1964). By helping a close relative reproduce, an individual transmits genes to the next generation, albeit indirectly. Hamilton (1964) pointed out that kin selection could occur via two mechanisms: (*a*) kin discrimination, when cooperation is preferentially directed toward relatives, and (*b*) limited dispersal (population viscosity), which keeps relatives in spatial proximity to one another, allowing cooperation to be directed indiscriminately toward all neighbors (who tend to be relatives). The clonal nature of most microbes raises some complications for the concept of genetic relatedness, which we discuss in Section 6. The other way to obtain an indirect fitness benefit is if cooperation is directed toward nonkin who share the same cooperative gene, that is, genetic relatives who are not necessarily genealogical relatives. This assortment or greenbeard mechanism requires a single gene (or a number of tightly linked genes) that both causes the cooperative behavior and can be recognized by other individuals owing to a distinctive phenotypic marker, such as a green beard (Dawkins 1976, Hamilton 1964).

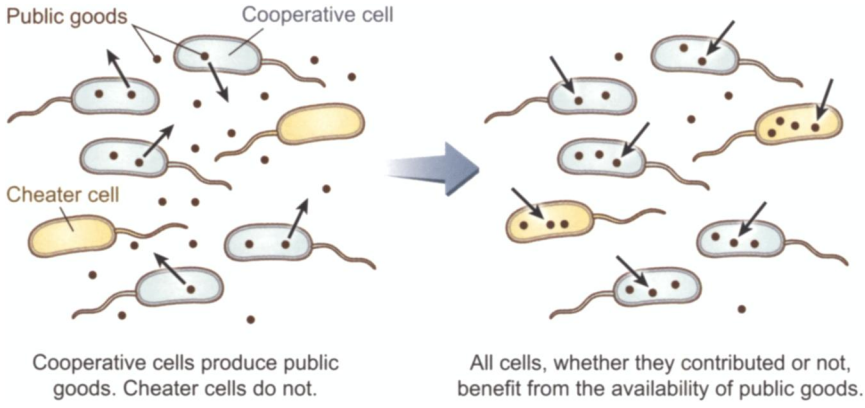

Figure 1

The tragedy of the commons with public goods. Cheats who do not pay the cost of producing public goods can still exploit the benefits of public goods produced by other cells.

3. MICROBIAL CASE STUDIES

3.1. Public Goods

Possibly the most common form of social behavior in microbes is the production of public goods. Public goods are products manufactured by an individual that can then be utilized by the individual or its neighbors (**Figure 1**). For example, bacteria produce numerous factors that are released into the environment beyond the cell membrane (**Table 1**). Public goods lead to the problem of cooperation because they are metabolically costly to the individual to produce but provide a benefit to all the individuals in the local group or population (West et al. 2006). Consequently, although the production of public goods can have direct fitness benefits to the individuals producing them, they can also have indirect fitness benefits to the individuals around them. The specific problem in this case is what stops the spread of cheats that do not produce public goods (or produce less) but benefit from those produced by others?

How can the social nature of a microbial behavior such as the production of some postulated potential good be determined (West et al. 2006)? A useful feature of working with microbes is that mutants who do not perform the behavior (termed cheats) often already exist, or can be constructed, or will evolve relatively quickly if they are selectively favored (Dugatkin et al. 2005, Foster et al. 2004, Griffin et al. 2004, Harrison & Buckling 2005, Rainey & Rainey 2003, Velicer et al. 2000). Once these are acquired, a first step is to examine the relative fitness of the wild type that performs the putative social behavior and the mutant that does not, when grown alone (monoculture) or in a mixture. This examines the social costs and benefits of the behavior. Such experiments require that the wild type and mutant can be distinguished when grown in a mixture. This can be done in a number of ways, including phenotypic features associated with the behavior (Griffin et al. 2004) or by insertion of a phenotypic or molecular marker (Foster et al. 2004, Mehdiabadi et al. 2006).

Table 1 Potential public goods

Public good	Role
Siderophores	Iron-scavenging molecules (West & Buckling 2003).
Invertase	An enzyme for digesting sucrose (Greig & Travisano 2004).
β-lactamase	Inactivates and therefore gives resistance to antibiotics (Ciofu et al. 2000)
Biosurfactants	Extracellular matrices for facilitating movement over surfaces, e.g., Rhamnolipid (*P. aeruginosa*) and Serrawettin (*Serratia marcescens*) (Daniels et al. 2004, Velicer & Yu 2003)
Exopolysaccharides such as alginate or adhesive polymers	Providing structure for growth, and the ability to colonize different habitats (Davies & Geesey 1995, Rainey & Rainey 2003)
Host-manipulation factors	Increasing host susceptibility to predation, immune suppression, host castration (Brown 1999)
Shiga toxins	Breaking down host tissue (O'Loughlin & Robins-Browne 2001)
Protein synthesis	Growth (Turner & Chao 1999)
Toxic and lytic secondary metabolites	To kill and degrade prey organisms
Adhesive polymer	Colonization of the air-liquid interface (Rainey & Rainey 2003)
Quorum-sensing molecules	Cell-cell signals (Williams et al. 2007), iron chelation (Diggle et al. 2007, Kaufmann et al. 2005), immune modulators (Pritchard et al. 2003), biosurfactants (Daniels et al. 2006), plant systemic resistance (Schuhegger et al. 2006)
Proteases	Extracellular protein digestion (Hase & Finkelstein 1993)
Extracellular DNA	Structural component of biofilms (Spoering & Gilmore 2006)
Antibiotics	To kill competitors (although better to conceptualize as spiteful rather than public good; see Section 4.6)
Membrane vesicles	Common biofilm component (Schooling & Beveridge 2006), transport of cell-to-cell signals (Mashburn & Whiteley 2005)
Rhamnolipids	Antiprotozoan defense mechanism (Cosson et al. 2002), mediate detachment from biofilms (Boles et al. 2005)
Microbial repellents	Repels competitors (Burgess et al. 2003)
Resources supplied by symbionts to their hosts, such as nitrogen fixation by rhizobia	Aids host growth and, in some cases, avoid enforcement by the host (Kiers et al. 2003, Kiers & van der Heijden 2006, West et al. 2002)

Recent research on siderophores provides an example of how this approach can be used to test the social nature of a potential public goods behavior. Siderophores are iron-scavenging molecules produced by many species of bacteria (Ratledge & Dover 2000, West & Buckling 2003). Iron is a major limiting factor for bacterial growth because most iron in the environment is in the insoluble Fe(III) form, and, in the context of bacterial parasites, is actively withheld by hosts. Recently, Griffin et al. (2004) investigated the social nature of the production of the siderophore pyoverdine in *P. aeruginosa*. Siderophore production is beneficial when iron is limiting, as shown by the fact that the wild type that produces siderophores outcompetes a mutant that does not, when the strains are grown in pure culture. However, siderophore production is also metabolically costly, as demonstrated by the fact that mutants outcompete wild-type strains in an iron-rich environment. Consequently, in mixed

populations where both wild-type and mutant bacteria are present, the mutants can gain the benefit of siderophore production without paying the cost, and hence increase in frequency.

The production of public goods such as siderophores could provide both a direct and indirect fitness benefit because they benefit the individual who produces them and their neighbors. This is an example of what has been termed a whole-group trait (Pepper 2000). Consequently, we would expect public goods to be subject to kin selection, with selection for higher levels of public goods production when there is higher relatedness between interacting individuals. An experimental evolution approach with siderophore production in *P. aeruginosa* (Griffin et al. 2004) provided support for the predicted effect of relatedness. Relatedness was manipulated by allowing the bacteria to grow and interact in groups derived from a single clone (relatively high relatedness) or from two clones (relatively low relatedness). The cooperative wild-type strain outcompeted the selfish mutant strain only when cultured under conditions of relatively high relatedness.

A useful feature of microbes is that they have provided excellent opportunities for testing how the relative cost and benefit of cooperation varies with population demographics such as density or frequency and environmental factors such as resource availability. For example, (*a*) at lower population density, cells will be less able to use the public goods produced by other cells and so cheats will do worse (Brown & Johnstone 2001, Greig & Travisano 2004), (*b*) cheats do better when they are at lower frequencies in the population (frequency dependence) because they are then better able to exploit cooperators (Dugatkin et al. 2003, 2005; Harrison et al. 2006; Ross-Gillespie et al. 2007; Velicer et al. 2000), (*c*) individuals would be expected to change their production of public goods depending upon the availability of resources in the environment, which will also be influenced by the behavior of their neighbors, and (*d*) competition between relatives reduces the kin-selected benefit of cooperation and hence favors reduced levels of cooperation (Griffin et al. 2004). The work in these areas stresses the importance of developing specific theory that can be tested with specific systems (Ross-Gillespie et al. 2007).

A relatively unexplored possibility in microbes is that selection would favor mechanisms that allow public goods to be preferentially directed toward closer relatives, analogous to kin discrimination. For example, with the production of public goods, selection would favor the production of highly specific molecules that other lineages (clones) could not utilize. Consistent with this, in *P. aeruginosa* there is variation across strains in the form of pyoverdine produced and in the ability of strains to uptake iron chelated by pyoverdines produced by other strains (Meyer et al. 1997). Furthermore, sequence data suggest that the genes involved in pyoverdine production are under selection for novelty and specificity (diversifying selection) (Smith et al. 2005).

We have focused on siderophores in this section because they provide a specific example where there have been several studies from an evolutionary perspective. However, as illustrated by **Table 1**, microbes produce a fascinating diversity of molecules that may function as public goods. There is enormous potential for future work in this area because, in practically all of these cases, even the most basic social nature of these traits has yet to be examined.

3.2. Fruiting Bodies

One of the most striking forms of cooperation in microbes is the development of fruiting bodies, as found in the cellular slime molds, or social amoebae, in which there is a huge diversity in the way fruiting bodies are formed (Bonner 1967). The most studied slime mold from this perspective is *Dictyostelium discoideum*, a predator of bacteria that is common in the soil. When starving, the usually solitary single-celled amoebae aggregate and form a multicellular slug (pseudoplasmodium, or grex) that can contain 10^4–10^6 cells. This slug migrates to the soil surface, where it transforms into a fruiting body composed of a spherical sorus of spores and a stalk consisting of nonviable stalk cells that hold the sorus aloft. Roughly 20% of the cells die to form the stalk in what appears to be an altruistic act. This division between nonviable stalk cells and spores leads to clear potential for conflict. If the slug is composed of a single lineage, with a mass of genetically identical cells, then kin selection would lead to no conflict over cell fates. However, when multiple lineages occur in a slug, each lineage is selected to make a relatively larger contribution to the spore cells and a relatively smaller contribution to the stalk, at a cost to the other lineages (Strassmann et al. 2000).

In *D. discoideum*, cells from distinct lineages will come together to form slugs and fruiting bodies, suggesting that the conflict exists in natural populations (Fortunato et al. 2003, Strassmann et al. 2000). Evidence for a struggle resulting from this conflict comes from the observation that, when mixed, different lineages show a variable ability to exploit other lineages in the race to provide a higher proportion of the spore cells (Fortunato et al. 2003, Strassmann et al. 2000). Furthermore, this conflict is costly, as shown by the fact that slugs formed by multiple lineages (chimeras with conflict) are not as good at moving as slugs formed by single lineages (where no conflict occurs) (Foster et al. 2002). Consequently, they are less able to migrate to a position where fruiting body formation is possible (Castillo et al. 2005). However, this effect is complicated by the fact that larger slugs are better at moving (Foster et al. 2002). Consequently, under certain conditions, it can be better to be in a larger chimera slug than in a smaller slug consisting of only one lineage (Foster et al. 2002). This suggests that we may expect variation in the allocation of resources to conflict across species that typically occur at different densities, or even facultative adjustment in response to local population density.

Another possible way to avoid the costs of forming chimeras would be if amoebae preferentially formed slugs with relatives (Mehdiabadi et al. 2006). Such kin discrimination has been observed in the species *D. purpureum* (Mehdiabadi et al. 2006). When pairs of clones were placed together in equal proportions at high density, in the absence of food, the slugs that were formed tended to be dominated by one or another of the strains rather than an equal mix. This led to the mean relatedness within slugs being approximately 0.8 rather than the expected of 0.5. A more extreme case has been found in *D. discoideum*, where a greenbeard mechanism preferentially directs cooperation toward individuals who share the same cooperative gene. Individuals who share the *csa* cell adhesion gene adhere to each other in aggregation streams—excluding mutants that do not share the gene—allowing them to cooperatively form fruiting bodies (Queller et al. 2003).

A system of differentiation analogous to the slime molds occurs in soil-dwelling myxobacteria. In *Myxococcus xanthus*, a lack of amino acids triggers aggregation into local groups that exchange intercellular signals and construct spore-bearing fruiting bodies. As in the *Dictyostelium* species, only a fraction of cells can become spores in these fruiting bodies, leading to the same conflicts of interest (Fiegna & Velicer 2006, Fiegna et al. 2006, Kadam & Velicer 2006). Research on *M. xanthus* has provided some elegant experiments on the costs and benefits of making fruiting bodies (Fiegna et al. 2006, Velicer et al. 2000). An important feature of this research is that it has utilized a number of cheater mutants that contribute less to the production of the nonspore parts of the fruiting body. The advantage of replicating the experiments with multiple mutants is that it demonstrates the generality of any results and that those results are not just due to a pleiotropic effect or correlated character. In addition, the mutant strains used here were constructed in two different ways: defined single gene mutants and selection under controlled laboratory conditions (Velicer et al. 2000). Each type of mutant cheater has its advantage. Strains that have had the gene for a cooperative behavior artificially knocked out have well-defined, clear, and large effects, whereas spontaneous mutations from the laboratory or isolates from the field may be more natural and may give a better indication of the kind of variation upon which natural selection could act.

4. OTHER SOCIAL BEHAVIORS

4.1. Resource Use

There can be a trade-off between the rate at which a resource can be used and the efficiency of its use (Kreft 2004, Kreft & Bonhoeffer 2005, MacLean & Gudelj 2006, Pfeiffer et al. 2001). For example, respiration of ATP leads to a higher energy yield than fermentation. Consequently, individuals that use both respiration and fermentation can produce energy at a higher rate but use the resources less efficiently than individuals that employ respiration only (Pfeiffer et al. 2001). Fermentation can therefore provide a direct benefit to the individual at a cost that is shared among the local population. This allows respiro-fermenters to outcompete respirers, leading to the familiar tragedy of the commons problem where the selfish interest of individuals leads to less efficient resource use (Hardin 1968). A solution to this problem, that can be important in microbes, is if interacting individuals are relatives, as this also leads to an indirect fitness cost of less efficient resource use (fermentation) by reducing the resources available for relatives. Consequently, more efficient resource use can be thought of as a cooperative behavior, with more efficient use favored by kin selection.

4.2. Quorum Sensing

Perhaps the paradigm for bacterial cooperation and communication can be seen in the diverse quorum sensing (QS) systems, which occur widely across bacteria (Williams et al. 2007). QS describes the phenomenon whereby the accumulation of signaling molecules in the surrounding environment enables a single cell to assess the number

of bacteria (cell density) so that the population as a whole can make a coordinated response. In many situations, a group venture is not worth taking on unless there is a sufficient number of collaborators to make it worthwhile. QS systems have been shown to regulate the expression of genes involved in plasmid transfer, bioluminescence, population mobility, biofilm maturation, and virulence (Williams et al. 2007). Many of the behaviors regulated by QS appear to be cooperative, for example, producing public goods such as exoenzymes, biosurfactants, antibiotics, and exopolysaccharides (see **Table 1**).

Little attention has been given to the evolutionary implications of QS (Brown & Johnstone 2001). Microbiologists frequently assume that QS is readily selected for because it benefits the local group or population as a whole (Henke & Bassler 2004, Shapiro 1998). In contrast, evolutionary theory suggests that cooperative communication is maintained only by selection under fairly restrictive conditions (Maynard Smith & Harper 2003). This raises the question as to whether QS in microbes is truly a cooperative behavior (Diggle et al. 2007, Keller & Surette 2006, Redfield 2002). Are QS molecules true signals? The fact that a compound produced by cell A elicits a response in cell B does not necessarily mean that there is signaling between the cells; it may represent cell B using the molecule as an environmental cue to guide future action, or it may represent cell A coercing cell B into a certain behavior. We suspect that within species, QS signaling between relatives will be favored by kin selection (Brown & Johnstone 2001, Diggle et al. 2007). In contrast, between-species QS (sometimes termed bacterial cross talk) is more problematic to explain and it could be that, in these cases, QS molecules are used by other species as cues or for coercion.

A fundamental first step is to determine the fitness consequences of producing and responding to a signal (Brown & Johnstone 2001). Although there is undoubtedly a metabolic cost for signal production (Keller & Surette 2006), it is likely that the cost of responding is far more expensive metabolically. For example, as mentioned above, 6%–10% of *P. aeruginosa* genes appear to be QS-regulated. Given high costs, QS signaling or response could be potentially exploitable by QS cheats (Diggle et al. 2007, Keller & Surette 2006). In theory, QS cheats could take the form of either (*a*) a signal-negative (mute) strain that does not make the molecule but can respond to it, or (*b*) a signal-blind (deaf) strain that may (or may not) make a signal but, more importantly, does not respond to it. Both types of mutants can be constructed, and signal-blind mutants are often isolated from clinical infections of *P. aeruginosa* (Smith et al. 2006).

Signaling in bacteria has a number of complexities that offer novel problems from an evolutionary perspective. First, the signal can be degraded. *N*-acyl homoserine lactone (AHL) signals are rendered biologically inactive in alkaline environments (Yates et al. 2002). Therefore, in certain environmental niches, signaling may be ineffective or the level and response to QS may vary. AHLs can also be degraded by enzymes produced by bacteria, a process known as quorum quenching (Dong & Zhang 2005, Sio et al. 2006). Can this behavior be considered coercive or spiteful, and are there indirect or direct fitness benefits for the AHL degrader?

Second, the genes required for signal generation (*luxI* homologs) and response (*luxR* homologs) are not always found on the bacterial chromosome. A number of

these homologs have been identified on plasmids such as the *Agrobacterium* Ti plasmid (Zhang et al. 1993) and *Rhizobium* symbiotic plasmids (Smith 2001, Wisniewski-Dye & Downie 2002). Although this may just represent an easy way to obtain QS mechanisms, it could also be a mechanism by which signaling is forced onto a cell that does not contain the QS machinery, coercing it into cooperative behavior (Smith 2001). An issue here is that the interests of the bacteria and the plasmids may conflict. Third, a number of other roles have been assigned to QS molecules. QS molecules can function as public goods, for example, iron chelators (Diggle et al. 2007), immunomodulatory compounds (Pritchard et al. 2003), and biosurfactants (Daniels et al. 2006). QS compounds can also be harmful or spiteful; for example, the lantibiotics typified by lactococcal nisin and produced by *Lactococcus lactis* are potent bacteriocides against many bacteria (Dodd et al. 1996, Stein 2005). The consequences of QS signals having multiple functions must be explored theoretically, as it will alter the costs and benefits of signaling as well as how they vary with parameters such as relatedness (Diggle et al. 2007).

Remarkably, given the size of the literature on the subject, it remains to be conclusively demonstrated that QS systems are mechanisms for cell-to-cell communication. Redfield (2002) raised the possibility that QS molecules allow individual cells to determine how rapidly secreted molecules move away from the cell. This diffusion sensing could allow cells to regulate the secretion of public goods to minimize losses due to extracellular diffusion and mixing. A possibility here is that the production of these molecules may have initially evolved for one reason (e.g., diffusion sensing) but are now maintained for another (e.g., QS).

4.3. Biofilms

Traditionally, bacteria have been thought of as free-swimming planktonic organisms. However, most bacterial species are capable of forming structured multicellular communities known as biofilms (Kolter & Greenberg 2006). Biofilms are ubiquitous, being found in such diverse environments as dental plaques, wounds, rock surfaces, and at the bottom of rivers. They have a definite structure, including water channels, which may involve a number of different specialist cells, and they are often enclosed by an exopolysaccharide matrix, which can make them difficult to eradicate. For example, biofilm-growing cells are often significantly more resistant to antibiotics than free-living (planktonic) cells, and the matrix can also help protect bacterial cells against the host immune system during infection. The life cycle of a biofilm can be split up into distinct stages: (*a*) reversible attachment, often mediated by flagella and type IV pili, (*b*) irreversible attachment to a surface, (*c*) formation of microcolonies, (*d*) differentiation into intricate architectures, and (*e*) dispersal (**Figure 2**). Biofilms are of particular interest from an evolutionary perspective because the close proximity of individuals in a biofilm can make cooperation and communication particularly important.

Many forms of cooperation can be involved in the establishment and growth of a biofilm. First, there is the cooperative production of an extracellular matrix (ECM), which surrounds the biofilm and may be important in maintaining structure. There

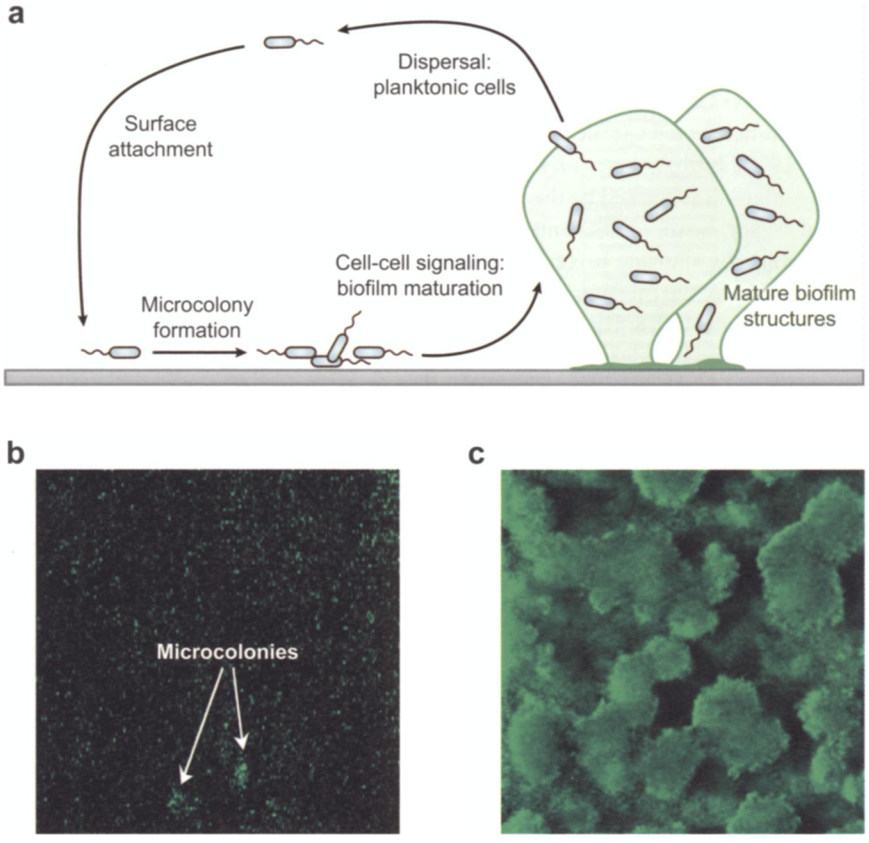

Figure 2

Life cycle of a bacterial biofilm. (*a*) Planktonic cells are released from mature biofilms and, via motility mechanisms, settle on a new surface. Cells become irreversibly attached and begin to form microcolonies. Mechanisms such as cell-cell signaling systems lead to the differentiation of mature biofilm structures. Diagram adapted from Kolter & Losick (1998). (*b*) Scanning electron microscopy image of *Pseudomonas aeruginosa* attachment to a stainless steel coupon. The formation of microcolonies can be observed. Image taken with permission from Diggle et al. (2006). (*c*) Scanning confocal microscopy image of mature five-day-old *P. aeruginosa* biofilms grown in flow cell chambers. Image courtesy of S. Crusz.

are many components found in the ECM that may be involved in holding the biofilm structure together, including alginate, the lectin LecA, and carbohydrate polymers (Davies & Geesey 1995, Diggle et al. 2006, Friedman & Kolter 2004, Matsukawa & Greenberg 2004, Nivens et al. 2001). Second, numerous other public goods can be important in biofilms, such as rhamnolipid, a biosurfactant that aids in biofilm detachment (Boles et al. 2005), and microvesicles, which are a component of the ECM and can contain signal molecules and proteases (Schooling & Beveridge 2006). Third, cell death can occur in a subpopulation of cells. Researchers have hypothesized that this is a cooperative behavior to release extracellular DNA that aids in

structuring the biofilm (Whitchurch et al. 2002). However, a complication here is that in *P. aeruginosa* cell death involves a genomic prophage (Webb et al. 2003). Consequently, this could be an altruistic behavior by the individual cells, or a selfish manipulation by a prophage. Fourth, dispersal from biofilms may be a social trait, if it has been selected to reduce competition with nondispersing relatives (Hamilton & May 1977). Fifth, QS may play an important role in the development and structuring of biofilms, as suggested by the poor biofilm formation of some QS mutants (Davies et al. 1998). Sixth, biofilms may also be important in protecting against protozoan predation, by allowing bacterial prey to reach a size beyond the maximum that can be handled by the predator, or through group-coordinated chemical warfare against the predator (Matz & Kjelleberg 2005).

When viewed under a confocal microscope, it is immediately obvious that biofilms are not simply random clusters of cells (**Figure 2**). Researchers have suggested that there may be some specialization within biofilms, analogous to how caste development in social insects allows individuals to specialize in certain behaviors. In *P. aeruginosa* biofilms, several phenotypically different cell variants that exhibited different behaviors have been isolated (Boles et al. 2004), in particular, a wrinkly variant that showed faster biofilm development, and greater resistance to stress, and a mini variant that showed greater dispersal from the biofilm. This variation is heritable, but variants can change back to the wild type or each other. Although it is tempting to think that this represents specialization favored by kin selection, there are alternatives. For example, a variant may spread selfishly within a biofilm at the expense of other types, even if this leads to a long-term decrease in the fitness of the biofilm or that lineage, analogous to how a cancer selfishly spreads within an individual, despite the long-term fitness consequences.

Kin selection would clearly be important in biofilms initiated by one or a small number of clonal lineages. However, naturally formed biofilms very rarely contain just one species of bacteria, let alone a single clonal lineage. For example, the colonization of human teeth and the oral mucosa can involve up to 500 species of bacteria (Kolenbrander et al. 2002). Nonetheless, kin selection may still be important in such cases if social interactions take place on a local scale (see also Section 6). For example, if the benefits of producing the materials that structure the biofilm, such as exopolysaccharides, or other public goods, are shared primarily with neighboring cells, then the clonal growth of bacteria means that these benefits can still be shared with close relatives (Xavier & Foster 2007). In this case, it may be useful to think of biofilms as consisting of a number of clonal lineages (groups of lineages), with cooperation primarily within lineages but competition primarily between lineages. Diversity may also promote cooperation in some situations because rarer and more specialized cooperators can be harder to exploit. Experiments on biofilm formation in *P. fluorescens*, where more diverse groups were found to be both more productive and less susceptible to invasion by cheats, have provided support for this (Brockhurst et al. 2006). A final complication is that cooperative interactions can occur between species within biofilms. For example, cooperation between *Streptococcus oralis* and *Actinomyces naeslundii* is important in the early colonization of human teeth, allowing these species to grow where neither can survive alone (Palmer et al. 2001).

4.4. Persisters

In populations of microbes, researchers have discovered that, at any point in time, a small proportion of cells do not grow at the normal rate but exist in a quiescent, nongrowing state (Lewis 2007). These cells are sometimes known as persister cells because they persist in the face of catastrophes such as antibiotic treatment (Cozens et al. 1986). Persister cells are resistant to antibiotics because antibiotics rely on disrupting the translation of the mRNA code to polypeptide chains, and this process does not occur in nongrowing cells. Researchers have suggested that persister cells are responsible for the recalcitrance of microbial populations in biofilms rather than, as is often assumed, the production of a protective ECM (Lewis 2007). Two important aspects of persister biology are that (*a*) the cells appear not to be genetically resistant to treatment by antibiotics—following antibiotic treatment, persisters give rise to new populations that have the same vulnerability to antibiotic treatment; and (*b*) persistence is controlled by a phenotypic switch—all cells have the potential to become persisters and persisters readily revert to normal growth rate (Balaban et al. 2004, Kussell et al. 2005, Lewis 2007).

In the microbiology literature, the general assumption has been that persistence is favored because it provides a benefit at the population level (reviewed by A. Gardner, S.A. West & A.S. Griffin, submitted). In contrast, a recent theoretical analysis suggests that persistence is better understood as a potentially social trait that can have both direct and indirect fitness consequences (A. Gardner, S.A. West & A.S. Griffin, submitted). Persistence provides a direct benefit by producing a phenotype that survives catastrophes, but it is also costly because it reduces the short-term growth rate. However, this reduced growth rate also reduces local competition for resources and hence provides an indirect benefit to relatives competing for the same resources. This means that higher levels of persistence are predicted with a higher relatedness between competing bacteria. Theory also predicts that a higher level of persistence will be favored when there is greater competition for resources. Data from clinical infections support this prediction, where there appears to be higher levels of persistence in populations that have entered the stationary phase owing to resource depletion (A. Gardner, S.A. West & A.S. Griffin, submitted). A novel feature of selection on persistence is that this last prediction and pattern are in a direction opposite to the usual prediction, that more local competition selects against cooperation (West et al. 2002).

4.5. Cell Death

Researchers have suggested that programmed cell death (PCD; also known as apoptosis or autolysis) is adaptive in several situations. PCD clearly provides no direct fitness benefit, and so explanations must rely on indirect benefits for relatives or cells being forced into cell death by others. Several altruistic possibilities have been suggested, such as providing resources that could be used by other cells for growth and survival in yeast (Fabrizio et al. 2006, Gourlay et al. 2006) or the formation of fruiting bodies in *M. xanthus* (Wireman & Dworkin 1977). In biofilms, PCD may also be useful

for (*a*) creating channels within biofilms, which are responsible for the transport of nutrients and waste to and from cells deep in the biofilm or through which cells can disperse, and (*b*) releasing extracellular DNA that can be used for structuring biofilms (Allesen-Holm et al. 2006, Webb et al. 2003, Whitchurch et al. 2002). QS may be involved in controlling PCD (D'Argenio et al. 2002).

If PCD is occurring as a form of adaptive suicide, then this is clearly an area where microbes differ from animals, where there is a relative lack of such behaviors. Charlesworth (1978) noted that it is extremely hard for a gene causing suicide to spread because only relatives that do not share the gene would benefit. The possible solution to this problem in microbes is that selection could favor a low probability of PCD among a large population of cells, possibly depending upon individual condition, environmental conditions, or signaling. However, we stress that the adaptive nature of altruistic PCD remains to be demonstrated empirically, and alternative explanations exist. For example, in *P. aeruginosa*, cell death is mediated by a bacteriophage (Webb et al. 2003), and although this may reflect host control (D'Argenio et al. 2002), there is also the possibility that the bacteriophage may favor host death under the low growth conditions found in biofilms, to aid their own transmission.

4.6. Bacteriocins

Bacteriocins are the most abundant of a range of antimicrobial compounds facultatively produced by bacteria and are found in all major bacterial lineages (Riley & Wertz 2002). They are a diverse family of proteins with a variety of antimicrobial killing activities, many of which can be produced by a single bacterium, including enzyme inhibition, nuclease activity, and pore formation in cell membranes (Reeves 1972, Riley & Wertz 2002). Bacteriocin production is spiteful because it is costly to the individual cell that performs it and also costly to the recipient, which is killed (Gardner et al. 2004). The individual cost of bacteriocin production may be the diversion of resources from other cellular functions. However, in many gram-negative bacteria, such as *Escherichia coli*, cell death is required for the release of bacteriocins (Chao & Levin 1981, Riley & Wertz 2002). Individuals from the same lineage (clone mates) are protected from the toxic effects of bacteriocins as a result of genetic linkage between the bacteriocin gene and an immunity gene that encodes a factor that deactivates the bacteriocin (Riley & Wertz 2002). Bacteriocin production can therefore provide an indirect fitness benefit by reducing the level of competition experienced by relatives and hence can also be viewed as indirect altruism. Maximum bacteriocin production should be favored at intermediate levels of relatedness; when relatedness is extremely high, there are few susceptible competitors to attack, and when relatedness is extremely low, there are fewer relatives to enjoy the benefits of relaxed competition (Gardner et al. 2004).

Given the strong selective pressure imposed by bacteriocins, it is unsurprising that bacteriocin resistance readily evolves. As with bacteriocin production, such resistance carries pleiotropic fitness costs. Such costs are critical for coexistence (via negative-frequency-dependent selection) between bacteriocin-producing, sensitive, and resistant strains in spatially structured environments (Czaran & Hoekstra 2003, Czaran

et al. 2002, Kerr et al. 2002), as producer beats sensitive, sensitive beats resistant, and resistant beats producer (Kerr et al. 2002). Furthermore, costly resistance is also likely to explain why there is considerable diversity in bacteriocin types maintained within microbial species. It is likely to be too costly to be resistant to all bacteriocins at once, and the probability of resistance will be lowest with respect to rare bacteriocins. As such, rare bacteriocin alleles will increase in frequency, allowing diversity to be maintained. Another interesting complication that can occur with bacteriocins is that they may help disfavor siderophore cheats: A pyoverdin (siderophore) receptor (type II) of *P. aeruginosa* also acts as a receptor for a *P. aeruginosa* bacteriocin (Pyocin S3), and so a cheat that evolved from a different lineage is likely to be susceptible to the toxins produced by the population being exploited (Tümmler & Cornelis 2005).

5. VIRULENCE

An appreciation of the widespread importance of cooperative social behaviors in microbes is leading to a change in how evolutionary biologists think about parasite virulence. In the 1990s, researchers saw a huge explosion of evolutionary theory designed to predict the damage that a parasite should inflict on its host, termed parasite virulence (reviewed by Frank 1996). The underlying idea in this theory was that higher growth rates enhance parasite transmission rates but incur greater mortality for the host and hence reduce the total duration of infection, and this results in a trade-off between the transmission benefit and the host-mortality cost. This is a social problem because the optimal use of a host's resources will depend upon the relatedness between the parasites infecting a host (Frank 1996). If only one lineage infects a host (high relatedness), this selects for a prudent use of host resources. As multiple lineages infect a host (low relatedness), parasites are selected to use host resources before their competitors, and this selects for higher growth rates and hence a higher virulence. Consequently, a tragedy of the commons arises, where higher growth rates provide a benefit to the individual and the cost of higher host mortality is shared over the group, analogous to the evolution of resources discussed in Section 4.1.

However, despite huge amounts of theoretical attention, there is a lack of empirical support for these predictions (Read & Taylor 2001). One possible explanation is that the diverse social lives of microbes can also lead to different predictions (West & Buckling 2003). The above prediction assumes that parasites can regulate their own growth rate, to whatever level they choose. However, an alternative possibility is that parasite growth rates are limited by host measures and that cooperative behaviors play a key role in increasing growth rates within hosts (West & Buckling 2003). Many public goods produced by microbes play important roles in acquiring resources from hosts or evading immune responses (**Table 1**). Indeed, we have found that when microbiologists refer to virulence factors, they are usually discussing a cooperative public good. Consequently, cheats that do not perform cooperative behaviors, such as siderophore production, cause a lower virulence than cooperators (Harrison et al. 2006). This leads to the prediction that growth rates and virulence should be higher when relatedness between parasites is greater (Brown et al. 2002, West & Buckling

2003). This prediction is in the opposite direction from that predicted in the previous paragraph. It is even possible to make more complicated predictions: With spiteful behaviors such as bacteriocin production, we predict the lowest growth rates and virulence at intermediate levels of relatedness because it is here that bacteriocin-mediated killing of bacterial cells is most favored (Gardner et al. 2004). The possible role of bacteriocins has been supported by experiments showing that infections of caterpillars with multiple species of bacteria that can kill each other lead to reduced virulence, compared with single-species infections (Massey et al. 2004).

6. RELATEDNESS

In the above sections we used the term relatedness rather informally. Social biologists familiar with diploid eukaryotes will be familiar with the classic results that relatedness between full sibs is $r = 0.5$ and between half-sibs is $r = 0.25$, in the absence of inbreeding (Hamilton 1964). Formally, relatedness describes the statistical association (regression) between genes in different individuals (Frank 1998, Hamilton 1970). In principle, the concept of relatedness is also clear in microbes. In the simplest case, if N unrelated lineages (clones) mix equally in a social arena, then average relatedness will be $r = 1/N$ (West & Buckling 2003). This comes from the average of individuals being related by $r = 1$ to their clonemates, and by $r = 0$ to individuals in the other lineages (assuming they are chosen randomly from the global population). However, several complications can occur, making the application of relatedness to microbial social behaviors less clear.

First, the relevant relatedness should be measured over the scale at which social interactions occur (West & Buckling 2003, West et al. 2006). This will vary between species and even between traits within species. For example, the relevant scale is (*a*) the distance over which public goods disperse when considering public goods such as siderophores (Section 3.1), (*b*) the whole fruiting body when considering cooperative stalk formation in *Dictyostelium* species (Section 3.2), (*c*) the whole host when considering symbiotic cooperation through partner fidelity feedback or how growth rates may be adjusted in response to their effect on host mortality (virulence), and (*d*) the scale at which enforcement occurs to maintain cooperation by symbionts (e.g., individual root nodules).

Consequently, when social interactions take place over a limited spatial scale, increased genetic variability within a patch will not necessarily lead to a decrease in relatedness. This is illustrated by considering biofilms, where clonal growth and low dispersal can lead to the biofilm consisting of a number of competing lineages (Xavier & Foster 2007). With respect to public goods such as exopolysaccharides, which provide a benefit over a scale smaller than the whole biofilm, the recipients of the social behavior can be strongly related to the actor even when the biofilm consists of lots of lineages or even multiple species. The scale at which interactions occur will vary in nature and could be manipulated experimentally, through factors such as shaken or unshaken liquid cultures, agar plates with a variable agar concentration, or in vivo. If selection can influence the scale of interaction, for example, by altering the diffusibility of public goods molecules, a number of questions arise, including whether

harmful products such as bacteriocins tend to be dispersed over larger distances than helpful products such as siderophores or whether the dispersal distance of different QS signals correlates with the kind of behaviors they trigger.

Second, the rapid generation time of microbes means that mutations can have appreciable effects on relatedness. Consider a population of clones that perform a cooperative behavior favored by kin selection, in which a mutant that does not perform this behavior arises. The relatedness between these mutants and the ancestral strain would be $r = 0$ (for reasons described above). Over time, mutation could therefore lead to a reduction in relatedness, allowing selfish cheaters to spread, and to the breakdown of cooperation. This suggests that the stability of cooperation will be influenced by factors such as mutation rates, patch lifetimes, and dispersal rates (Michod & Roze 2001). West et al. (2006) have previously suggested that mutation in populations with low disperal provides a possible explanation for why cooperative traits such as QS signaling and protease production decline over time in long-term *P. aeruginosa* infections (Lee et al. 2005). If mutation rates are important, this also suggests the possibility for selection on mutation rates of social traits, as shown by the fact that cheat mutants that do not produce siderophores can arise and spread faster in populations with an elevated mutation rate (Harrison & Buckling 2005).

Third, genes for cooperative behaviors can sometimes be transmitted horizontally between different bacterial lineages, by mobile genetic elements such as conjugative plasmids or lysogenic phages (Smith 2001). Smith (2001) suggested that this can be a way of stopping the spread of cheats within a population by reinfecting them with cooperative behavior. This fascinating possibility requires further theoretical and empirical exploration. It would be useful to model the coevolutionary dynamics from the perspective of all the parties involved: donor, recipient, and the mobile genetic element. For example, the extent to which transfer could be counteracted or aided by selection to avoid or uptake such genetic elements is not clear. This emphasizes that, when determining the importance of kin selection, what matters is similarity at the gene controlling the social behavior (Dawkins 1979, Grafen 1985). In animals, we are accustomed to estimating this with pedigrees because here relatedness is (on average) the same throughout most of the genome, whereas in microorganisms we may have to get used to focusing on the actual gene.

A general point here is that the application of social evolution theory to microbes offers some novel problems compared with the traditional social evolution study of organisms such as insects and birds. In particular, although the same social evolution theory should apply to both macroscopic and microscopic organisms, the details can be different, with the important factors varying in very general ways. For example, considering kin selection, (*a*) limited dispersal may be the key mechanism in microorganisms (Griffin et al. 2004, West et al. 2006), whereas kin discrimination is the key mechanism in macroorganisms such as cooperative breeding vertebrates (Griffin & West 2003), (*b*) greenbeard effects may be more important in microorganisms because the required signaling and recognition can take place at the level of individual cells interacting with their neighbors (Queller et al. 2003), (*c*) spite may be more common in microorganisms owing to local competition for resources and extreme differences

in relatedness (e.g., clonal versus unrelated, with nothing in between) (Gardner et al. 2004), and (*d*) adaptive suicide could be occurring in microorganisms.

7. CONCLUSIONS

To a large extent, the field of social evolution in microbes is still relatively wide open. There are a huge number of behaviors and traits that are likely to be social but that have not been examined theoretically or empirically from an evolutionary perspective. Much of the early evolutionary work in this area was aimed at the basics, such as showing that cheats or conflicts of interest could occur (e.g., Strassmann et al. 2000, Velicer et al. 2000). Although similar studies are still required for a huge range of traits, there are also possibilities for using microbes to test specific predictions of social evolution theory (Griffin et al. 2004) or for clarifying the complexities that can occur in microbes (Section 6). Microbiologists have given these evolutionary issues little attention because (*a*) their focus is on the underlying mechanisms and genetic control of behaviors, and (*b*) it is frequently assumed that cooperative behaviors can be explained by species or even community-level benefits (i.e., cooperation is not a problem and conflict does not occur). Future progress will be maximized by interdisciplinary exchange between microbiologists, evolutionary biologists, and ecologists.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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