Sociobiology of the Myxobacteria

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Abstract

Cooperation is integral to much of biological life but can be threatened by selfish evolutionary strategies. Diverse cooperative traits have evolved among microbes, but particularly sophisticated forms of sociality have arisen in the myxobacteria, including group motility and multicellular fruiting body development. Myxobacterial cooperation has succeeded against socially destructive cheaters and can readily re-evolve from some socially defective genotypes. However, social harmony does not extend far. Spatially structured natural populations of the model species Myxococcus xanthus have fragmented into a large number of socially incompatible genotypes that exclude, exploit, and/or antagonize one another, including genetically similar neighbors. Here, we briefly review basic social evolution concepts as they pertain to microbes, discuss potential benefits of myxobacterial social traits, highlight recent empirical studies of social evolution in M. xanthus, and consider their implications for how myxobacterial cooperation and conflict evolve in the wild.

Key Words

altruism, cheating, cooperation, conflict, kin selection, Myxococcus xanthus, social evolution, sociomicrobiology
INTRODUCTION

Cooperation—behavior that benefits the reproductive fitness of others—is prevalent throughout the biological world, both within and across species and at all levels from genes to societies (11, 70). However, explanation of successful cooperation and the major transitions in biological organization it has produced must account for the problem posed by selfish individuals that gain an immediate fitness advantage by failing to cooperate themselves while nonetheless exploiting the cooperation of others (2, 42, 63, 93, 126).

The challenges of identifying and characterizing cooperative traits in animals, determining who benefits from them and to what degree, and understanding the evolutionary mechanisms that maintain them have filled uncounted reams of evolutionary literature. However, presumably from long before animals first arose, microorganisms were already engaged in myriad forms of cooperation. An increasing number and diversity of microbial behaviors are known or proposed to be cooperative (8, 16, 110, 125), including the production of public goods such as iron-chelating siderophores (41), exoenzymes that digest complex growth substrates (40, 90) or disarm antibiotics (23), anticompetitor toxins (12), proteins required for replication by coinfecting viruses (20, 109), and quorum-sensing compounds (79). Microbes also cooperate during some forms of motility (58, 65, 117) and in the formation of biofilms (73, 74, 137), hyphae (14), and multicellular fruiting bodies (Figure 1) (100, 104).

The relevance for microbes of theory originally developed to explain animal social evolution has been recognized for decades (2, 12, 55, 140). Only recently, however, has the prevalence of sociality in the microbial world become widely appreciated and have explicit conceptual and theoretical connections between animal and microbial cooperation become common (16, 32, 56, 81, 108, 110, 125, 127). The field of sociomicrobiology (79) is growing rapidly, spurred by the recognition that social microbes (a) provide insight into the earliest forms of cooperation among reproductively distinct individuals; (b) profoundly affect many aspects of human welfare (29); (c) provide powerful experimental systems for addressing
basic questions about social evolution, because many variables that affect cooperation can be experimentally controlled [e.g., within-group relatedness (41), migration (57), mutation rate (44), density (51), and the costs and benefits of cooperation (23, 39)]; and (d) can reveal aspects of social evolution unique to microbes (101, 127). Microbial social evolution encompasses both interspecific (93) and intraspecific forms of cooperation, but here we focus on the latter.

**COOPERATION AND CONFLICT**

Cooperative behaviors can be classified with respect to who they benefit (125). Mutually

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**Cooperation:** behavior that increases the fitness of one or more recipient individuals
beneficial cooperation directly increases the reproductive fitness of an actor (an organism that performs a behavior) as well as recipients (individuals affected by an actor) (Table 1). Behaviors that are mutually beneficial in groups may or may not benefit those that perform them in isolation (110). For example, production of digestive exoenzymes needed for growth by Myxococcus xanthus can benefit isolated cells (71) and may also benefit both producers and their neighbors in some environments (90). Alternatively, some behaviors (e.g., production of quorum-sensing molecules) might only generate benefits in a social context.

In evolutionary parlance, altruistic behavior increases the fitness of others but decreases the fitness of the actor (Table 1). Cell death during fruiting body formation (104, 132) and as a result of toxin production (12) are extreme examples of microbial altruism, at least to the extent that surviving cells benefit from it. The exploitation of altruists, or the enhancement of one's evolutionary fitness by noncooperation (or reduced cooperation), is commonly referred to as selfishness or cheating.

Conflict occurs when social interactions generate fitness asymmetries between interactants. Consider a focal social process that is important for fitness at the group level (e.g., M. xanthus fruiting body development and sporulation) (Figures 1 and 2). Suppose that two genotypes interact during the social process and one gains an increase in fitness relative to the other from the interaction. Suppose further that the losing genotype is highly proficient at the focal behavior when it is alone in clonal groups. The winner from interaction in chimeric groups can be classified with respect to how well it performs alone in clonal groups, the effect of the interaction on the winner’s absolute performance (Figure 2), and the molecular mechanisms associated with it. Table 1 illustrates this by tabulating the fitness effect of cooperation.

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**Table 1 Fitness effects of cooperation**

<table>
<thead>
<tr>
<th>Type of cooperation performed by actor</th>
<th>Fitness effect on Actor</th>
<th>Fitness effect on Recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutually beneficial</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Altruistic</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
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**Figure 2**

Social conflict. Asymmetric fitness in chimeric groups of socially defective (a and b, red and blue, respectively) and socially proficient genotypes (green) and of (c) two socially proficient genotypes, one of which (yellow) beats the other (green) in chimeric groups. This figure is explained in terms of Myxococcus xanthus development, but many of its features apply generally across microbial cooperative traits. Circle size represents total group size (i.e., spore count) after development. Single-color circles (left and right columns) represent clonal groups, whereas mixed-color circles (center columns) represent chimeric groups. It is assumed that all groups (both chimeric and pure) were of equal total population size at the onset of development and that chimeric groups were formed by equal initial numbers of paired genotypes. Sector areas in chimeric circles represent competitor spore population sizes after development. In real populations, all variables (clonal group spore production, initial competitor ratio in chimeras, total spore production by chimeric groups, and relative spore production within chimeric groups) can vary continuously. Here, several qualitatively distinct outcomes of chimerism that have been observed in M. xanthus (26, 113, 121) are shown, although many other outcomes are possible. (a) Socially defective cheater. A genotype with an obligate social defect gains a fitness advantage in chimeric groups (red > green in chimeric circles) but has a large disadvantage in competition between pure groups of cheaters and cooperators (clonal red < clonal green). Chimerism can increase (upper chimeric circle), decrease (lower chimeric circle), or have no effect (not shown) on total group spore production (see also Figure 3). (b) Disadvantageous defection. Failure to cooperate due to an obligate social defect does not confer a fitness advantage relative to cooperators in chimeric groups (blue < green in chimeric circles), but chimerism nonetheless enhances the absolute performance of the defective genotype (clonal blue circle < blue sector in chimeric circle). (c) Socially proficient winner. A genotype (yellow) that performs equally well at a focal social trait as its competitor (green) in pure clonal groups (clonal yellow = clonal green) wins in chimeric groups (yellow > green in chimeric circles) with either an absolute increase or decrease in its spore production (top and bottom outcomes of chimerism, respectively).
that mediate the winner's interaction-specific advantage.

**Socially Defective Cheaters**

One important form of social conflict occurs when one genotype (say, R, corresponding to the red genotype in Figure 2a) wins in chimeric groups specifically because it is genetically defective at performing a focal cooperative trait (e.g., production of a signal molecule) in both clonal and chimeric social environments. When this occurs, genotype R is said to cheat in chimeric groups because R exploits the costly signal production of a cooperative genotype (say, G, corresponding to the green genotype in Figure 2a) for its own absolute and relative advantage while itself paying a reduced or zero cost of cooperation. In this scenario, defection from cooperation is genetically obligate.

### Defection: failure to cooperate
in all social contexts and R therefore performs poorly in clonal groups relative to G under selective conditions in which the focal trait is important for fitness. Such cheating is evolutionarily obligate if R requires opportunities to cheat in order to persist in a population (27). Evolutionarily obligate cheating in microbes can occur if the cheater’s defect is severe and if the focal cooperative trait is a major component of overall fitness. (See Online Supplement regarding semantic issues related to cheating; follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org).

When socially defective cheaters reach high frequencies in mixed groups, they impose a group cost, or cheating load (108). Such cheaters decrease the level of cooperation in a group, thereby lowering group productivity and the mean fitness of individuals in that group, to a degree proportional to both cheater frequency and the severity of a cheater’s defect in clonal groups (Figure 3) (92, 113). If a defect that mediates cheating is total, cheaters then pose a threat to the very existence of cooperation (25), and their increase in a population must somehow be limited for cooperation to persist. Many examples of cheating conferred by genetic defects in a variety of social traits have been documented across a wide range of microbial species (7, 13, 15, 20, 22, 23, 39, 40, 41, 43, 50, 66, 85, 94, 109, 122), including during the process of spore formation within multicellular fruiting bodies by M. xanthus (113) and the social amoeba Dictyostelium discoideum (36, 96), and many more should be discovered in the future. The fitness of socially defective cheaters in chimeric groups with cooperators is characterized by negative frequency dependence (Figure 3) (92).

In contrast to social defects that allow successful cheating, not all mutations that harm a social function are advantageous in the presence of socially proficient strains (Figure 2b) (30, 113). Such nonadvantageous defects are selected against in all social contexts, but opportunities to mix with strains that partially complement such defects will slow their rate of loss from a population.

Socially Proficient Winners

In contrast to the above scenario, two strains [yellow (Y) and green (G) in Figure 2c] might
perform equally well at a focal cooperative trait in clonal groups, but one strain (Y) nonetheless wins owing to interaction in mixed groups. In this scenario, the persistence of cooperation per se is not threatened because individuals of both competitor genotypes cooperate proficiently with their own kind (unlike socially defective cheaters; Figure 2a). One cooperator simply threatens to displace another and cooperation among kin persists regardless of who wins.

One way such a genotype (Y) might win from an interaction is for Y to somehow exploit the presence of G for an absolute gain in its own performance (e.g., at spore production) as well as for a relative advantage over G (Figure 2c, upper chimeric circle). Such exploitation of one socially proficient genotype by another has been referred to as facultative exploitation (26) and facultative cheating (96) and is less likely to be required for evolutionary persistence than is cheating by obligate defectors. Such a requirement would only occur if Y were outcompeted by G in some other trait (e.g., predation) and therefore required opportunities to exploit G at the focal cooperative trait to compensate.

Alternatively, one socially proficient genotype (again represented as Y) might win over another genotype (G) because of an interaction between them despite itself suffering a decrease in absolute performance (Figure 2c, lower chimeric circle). Such an outcome might result from both competitors producing distinct anticompetitor compounds (34) but with one competitor being harmed less by such warfare than the other. Interaction-specific fitness advantages by socially proficient genotypes have been associated with both gains and losses in the absolute performance of the winner in *M. xanthus* (26, 121).

Finally, fitness might be unaffected by encounters between genotypes. In this case, one genotype will win in chimeric groups only if it inherently performs better at the focal cooperative trait in clonal groups.

**The Stabilization of Microbial Cooperation**

For altruistic cooperation to thrive, cooperators must interact more with each other than they do with socially defective cheaters. In formal kin selection theory (see sidebar, Social Evolution Theory) (42), this condition is met when populations are structured so that genetically similar individuals sharing cooperation alleles preferentially interact (i.e., average relatedness among interactants is high). At maximum relatedness in microbial populations, cooperators and cheaters would be distributed in separate clonal groups, whereas at minimum relatedness they would be homogeneously distributed. Forces that can lower relatedness among interactants include spontaneous mutation of cooperators into cheaters, individual-level selection favoring cheaters over cooperators within mixed groups, and successful migration of cheaters across social groups. Mechanisms limiting these forces that undermine cooperation can operate at the genetic or behavioral level or can be passively imposed by environmental spatial structure (108, 126).

**Limitation of the Mutation Rate to Cheaters**

At the genetic level, the rate of mutation to cheater phenotypes can be limited by the evolution of genetic networks that pleiotropically link expression of a cooperative trait to performance at some other component of fitness (30, 108). When this occurs, mutations causing a defect in a social trait might simply fail to confer any cheating advantage at all in the presence of cooperators (Figure 2b) (113). Alternatively, social defect mutations might generate cheaters that increase transiently within a group during one life cycle phase (e.g., sporulation in *M. xanthus*) but decrease to an even greater degree during a different phase (e.g., predatory swarming in *M. xanthus*) owing to antagonistic pleiotropy. Only cheaters with social defect mutations that confer a net within-group
SOCIAL EVOLUTION THEORY

Conditions allowing alleles that promote altruism to be favored by natural selection can be represented mathematically. This is most commonly done in the framework of kin selection theory (also known as inclusive fitness theory), which was initiated by William Hamilton (42) and is now formulated in terms of four major factors: the direct fitness cost of altruism to the actor (quantified by the parameter $c$), the fitness benefit of altruism gained by recipients (parameter $b$), the average degree of relatedness (i.e., genetic similarity) among individuals that interact during cooperation (parameter $r$), and the degree to which kin compete nonrandomly with one another for local resources (81, 128). The first three factors constitute the parameters of Hamilton’s famous kin selection inequality, which stipulates that altruism will be selectively favored if $rb - c > 0$ (42). This equation captures the inclusive fitness effect of altruism because it not only incorporates the direct (negative) effect of altruism on the actor ($c$), but also includes the combined indirect benefit conferred on all recipients that share an altruism allele with the actor ($rb$). When the latter is greater than the former, altruism should increase.

The degree of competition among kin, which is determined by the spatial scale of competition, also influences the selective fate of altruism (81, 128). If individuals compete, as well as cooperate, nonrandomly with kin because of limited dispersal, the scale of competition is said to be local. Inversely, as the scale of competition increases (i.e., becomes more global), competition becomes random with respect to kinship. When competition is local, the negative effects of kin competition counteract, and might entirely negate, the benefits of kin cooperation. Cooperative behaviors promoting population expansion (e.g., digestive exoenzyme production by microbes) are expected to be less constrained by kin competition than traits that do not owing to a greater cooperation benefit (81).

Altruistic cooperation is therefore most strongly favored if its benefit is large, its cost is small, relatedness among interactants during cooperation is high, and the scale of competition is global. Mutation to noncooperation alleles, within-group increase of cheaters, and migration of cheaters across social groups all decrease relatedness and therefore the overall level of cooperation in a population. A recent study with *D. discoideum* has initiated efforts to explicitly estimate relatedness within natural populations of social microbes (36).

The evolution of altruistic traits can also be formally modeled in terms of the relative effects of selection within versus between populations of social microbes (36). Among-group selection occurs, groups with high frequencies of cooperators will contribute more individuals to the subsequent generation than groups burdened by a high cheating load (Figure 3a), and alleles for cooperation can increase overall in a population even while they decrease within chimeric groups (13, 36, 41). For example, consider a simple population composed of three groups, the two clonal groups of *M. xanthus* cells represented in Figure 2a and the smaller chimeric group. Prior to selection for cooperative spore production, the frequencies of the two represented competitors averaged across all three groups were 0.5 (not shown graphically). However, after selection (Figure 2a) the cheater genotype has decreased to only a small fraction of the total three-group population, even though its frequency increased greatly within the chimeric group.

When cheaters are rare within a focal group, they increase in frequency without substantially reducing overall group productivity. [Counterintuitively, some *M. xanthus* cheaters can even increase group productivity at some frequencies (Figure 3a, red squares)]. Rare cheaters therefore have a fitness advantage both over cooperators in their own group and over cooperators in other, cheater-free groups (Figure 3b, red circles and blue squares, respectively). However, above a threshold cheater frequency, cheating load will reduce the productivity of a group sufficiently so that cheaters within that group have a lower population-level fitness than cooperators in other, cheater-free groups, even if they retain a fitness advantage over cooperators within their own group (Figure 3b) (36).

(Continued)
Limitation of Migration

Migration across social groups can promote cheater expansion by providing new opportunities to cheat (i.e., access to previously unexploited groups of cooperators). Migration lowers relatedness and thus decreases the variation between groups necessary to allow among-group selection to counteract selection at the individual level within groups. If intergroup migration is sufficiently low (and average relatedness correspondingly high), socially defective cheaters that arise by mutation and increase within their group of origin can nonetheless subsequently decrease in the overall population because of cheating load (36). Migration that allows cheaters to exploit new groups requires translocation to at least the perimeter region of the new groups in all cases and successful physical infiltration of those groups in some cases. (Diffusible cooperation molecules might be exploited at some distance from producer cells.) In principle, migration can be limited simply by environmental spatial structure if physical barriers prevent translocation from one group to another. In microbes, however, active motility and/or external vectors (e.g., water and insects) should often cause cells to encounter new social groups and facilitate cheater spread. Once cheater cells reach the perimeter of a new group of cooperators, successful infiltration of the group is necessary for cheating to occur on cooperative traits that require close cell-cell proximity (e.g., fruiting body formation). When environmental spatial structure is insufficient to prevent cheater spread, there may be selection for cooperators to generally exclude distinct genotypes from joining their groups (78, 108, 121).

Limitation of Proximate Cheaters by Cooperator Phenotypes

Cooperative microbes might evolve behavioral phenotypes that prevent defectors within their group from effectively cheating (27, 108). At least in theory, cooperative microbes might specifically withhold cooperation from defectors or even actively punish them. Such antichecking (or policing) behaviors that require specific discrimination of cheaters are common in animals (6, 28) but may be difficult for microbes to evolve. Cooperation among microbes might also be enforced by the transfer of cooperation genes into defectors via mobile genetic elements (101). Finally, if cooperation occurs through contact between adhesin molecules present on the surface of interacting cells, then nonproducers of the adhesin are inherently excluded from the benefit of cooperation (84, 102).

The overall population-level frequency of cheaters will be determined by a complex combination of the forces that promote cheating (i.e., mutation to cheater phenotypes, cheater advantage within groups and migration) and those that limit it [e.g., any within-group disadvantage cheaters have at high frequency (Figure 3b, red line) (39, 113), selection among groups with differential cheating load, and any cooperator phenotypes that hinder cheaters], as well as genetic drift.

MYXOBACTERIA

The myxobacteria (Deltaproteobacteria, order Myxococcales) include several dozen identified species and are best known for their construction of spore-bearing fruiting bodies in response to starvation (Figure 1) (100). Myxobacteria are prevalent throughout terrestrial soils but can be isolated from a wide variety of environments (18, 88, 89, 100), including freshwater and saltwater, tree bark, animal dung, and anaerobic (95) and hypothermic (19) habitats. Many species of myxobacteria collectively kill and degrade other microbial species as prey by secreting antibiotics and exoenzymes (3, 91). Myxobacteria produce a vast array of
secondary metabolites of unknown function (5) that may play roles in predation and competition (26). Previous work has provided important insight into the biogeography of myxobacterial diversity (18), but new technologies for genomic characterization of microbial community composition employed in broadscale surveys (in concert with traditional isolation methods) should greatly expand our knowledge in this regard.

Fruiting body morphologies vary dramatically across species (Figure 1) (18, 100, 105), but the adaptive significance of this variation is unclear. Like metazoan development, fruiting body formation is regulated by complex genetic networks and multiple stage-specific intercellular signals (99). Unlike animal development, multicellular development in the myxobacteria represents the aggregation of reproductively independent individuals into cooperative social groups rather than the unfolding of a multicellular entity from a single cell. Thus, myxobacterial fruiting bodies can be chimeric from their origin if multiple genotypes coaggregate.

Traditional myxobacterial taxonomy based on fruiting body and cell phenotypes (88, 100) has been largely supported by early DNA-sequence-based phylogenies, at least above the species level (103). Whole-genome-based phylogenies from many species should soon be generated, thereby allowing species relationships and social gene evolution (37) to be thoroughly examined. Myxobacterial genomes are large (9–13 Mb) and encode unusually large numbers of genes involved in two-component signalling systems (130) and secondary metabolite synthesis pathways (38, 97).

**Myxococcus xanthus**

Most research on the genetic and molecular basis of myxobacterial sociality has focused on the model species *M. xanthus*, which forms relatively simple fruiting bodies (Figure 1). *M. xanthus* development is initiated by an unusual quorum-sensing system that is triggered by amino acid depletion (54). Development terminates with the differentiation of rod-shaped cells into spherical spores within the fruiting body (Figure 1). However, many cells do not become spores (>90% under some conditions) and either undergo autolytic cell death (75, 132) or remain rod-shaped cells that circle the fruiting body perimeter (76). Population reduction within fruiting bodies imposes strong selection on sporulation efficiency within chimeric groups.

*M. xanthus* collectively preys upon a broad range of microorganisms (3, 91). Diffusible predation molecules are public goods that might be exploited by selfish genotypes (110), but it remains unclear whether effective cheating during predation does in fact occur. *Myxococcus* predation is facilitated by gliding movement across solid surfaces generated by two genetically distinct but synergistic motility systems (47). The S-motility (or social motility) system is obligately social and is driven by Type IV pili (133) that retract after contact with components of the *M. xanthus* exopolysaccharide (EPS) matrix (65) (previously known as the fibril matrix). S-motility in *M. xanthus* is similar to Type IV pili-based twitching motility in bacterial species such as *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae* (69) except that it requires cell-cell contact to function. The less understood A-motility (or adventurous motility) system (72, 139) allows the movement of isolated cells but is nonetheless normally employed in a group context.

Extensive genetic, biochemical, and behavioral characterization; molecular genetic tools; a sequenced genome; and ease of culture make *M. xanthus* an attractive model system for the study of microbial cooperation and its evolution in both laboratory and natural populations. Evolving laboratory populations descended from a clonal ancestor rapidly evolve striking changes in behavioral phenotypes due to selection on spontaneous mutants (27, 46, 112, 113, 117). For example, in less than 300 generations, laboratory populations of *M. xanthus* that evolved under conditions in which patches of prey (*Escherichia coli*) were scarce gained severalfold improvements in their ability to search for new patches (46).
WHY LIVE IN GROUPS?

Understanding the nature of cooperative traits and exactly how microbes benefit from them is challenging (21, 56, 73, 87, 108, 136). In the myxobacteria, the precise benefits derived from cooperation in natural habitats remain largely unclear (111).

For many microbes, group living is an obligate affair, simply because intimate proximity among cells growing by binary fission in structured habitats is unavoidable. Traits that make the best of such forced proximity by generating fitness benefits from cell-cell interactions will be favored by selection. If cells can actively leave groups, however, social congregation will be favored only if it generates fitness benefits inaccessible to isolated individuals. *M. xanthus* cells can move in isolation by A-motility (48) and hence have the potential to migrate away from groups. However, most natural isolates produce adhesive cell surface compounds and slime tracks that hinder cells from migrating alone and most cells that do wander from a group tend to return (47, 48, 61). Why does group living in *M. xanthus* persist when actively opting out of social life is possible?

Stress Protection During Growth

Primitive benefits of group living must have preceded the evolution of complex fruiting body development. Most simply, high cell density might somehow protect against various environmental stresses and thereby enhance growth or survival rate (64, 107, 131). Unpublished results indicate that *M. xanthus* growth rate is strongly density dependent under high acid stress but markedly less so at neutral pH (H. Peitz & G.J. Velicer, unpublished data). Protection against stress during growth may thus be a foundational benefit of group living in the myxobacteria.

Wolf-Pack Predation

Another basal benefit of social life may derive from the collective [or wolf-pack (24)] manner in which many myxobacterial species kill and hydrolyze other microbes (17, 31) by secreting a slew of biocidal compounds (91). High cell density may generally enhance growth on prey (90) or macromolecules that require extracellular degradation [e.g., cellulose (111, 138)] by generating a high local concentration of lytic compounds. In support of this hypothesis, growth of *M. xanthus* on nonhydrolyzed polypeptides in liquid medium is density dependent (90). To our knowledge, density-dependent growth of myxobacteria on prey cells in a structured habitat has not been reported.

Social Motility

The two motility systems of *M. xanthus* allow cells to actively migrate in search of new food sources and aggregate into fruiting bodies upon starvation, but why maintain two systems rather than only one? First, on most surface types that have been examined, A- and S-motility interact synergistically to enhance swarming rate relative to the use of only one system (45, 98). Second, S-motility promotes the tight packing of spores into *Myxococcus* fruiting bodies (135), which may be beneficial in several respects. Finally, the presence of two motility systems allows effective swarming over a broader range of surface types than does either system alone (45, 98).

Rising Together

Why sporulate en masse within elevated fruiting bodies rather than as isolated individuals? Complex development is not inherently required for sporulation, as some *M. xanthus* genotypes (112) and some myxobacterial species do not make distinctive fruiting bodies (49, 100). Isolation methods are often biased toward the recovery of fruiting genotypes (100) and many uncharacterized nonfruiting myxobacteria may exist (114). Indeed, the anaerobic species *Anaeromyxobacter dehalogenans* appears to have lost not only the ability to form fruiting bodies but also the ability to make any spores at all (106). Nonetheless, many species do construct complex fruiting
structures and spore production in *M. xanthus* is highly density dependent (51, 62), indicating that becoming a spore in a social context is often beneficial.

A frequently invoked potential benefit of fruiting body formation is enhanced dispersal to habitats favorable for growth (53). This hypothesis is most plausible for species with tall fruiting bodies or species that undergo development in response to light (129), but its relevance for species with short developmental aggregates is uncertain and its actual veracity for any species is unknown. A second major hypothesis about fruiting body benefits is that high spore density increases germination and growth rates when fruiting bodies encounter fresh patches of growth substrates (53, 90, 100). Taken together, these hypotheses suggest that myxobacterial fruiting body formation may represent a budding strategy that reduces local competition among kin by promoting dispersal but nonetheless allows small groups of kin to disperse together and thereby retain the benefits of kin association during cooperation (33). Sporulation within fruiting bodies might also produce higher-quality spores than would individualistic sporulation (132), and the extracellular matrix in which spores are embedded may protect against various external threats (e.g., predation or anticompetitor compounds) or abiotic stresses (124).

**WHY COOPERATE?**

A cooperative trait that is favorable in some environments can be lost if selection is relaxed by environmental change or dispersal to new habitats. More interestingly, even when cooperation is advantageous at the group level it will decrease within groups when socially defective cheaters appear by mutation or immigration. Both of these threats to the maintenance of cooperation are readily manifested in laboratory populations of *M. xanthus* (25, 46, 112, 113).

**Lost in Luxury**

The fate of cooperation under relaxed selection was examined among replicate populations of *M. xanthus* derived from a clonal ancestor that evolved in nutrient-rich liquid for 1000 generations (112). In this environment, motility, predation, and development were all unimportant for fitness. All populations evolved to grow faster but also rapidly (in as few as 200–300 generations; G.J. Velicer, unpublished data) incurred severe defects in S-motility and development. The uniformity and speed of social loss across populations indicate that the mutant alleles causing these losses were favored by selection. *Myxococcus* sociality can also fare poorly under less luxurious conditions. Other experimental populations underwent cyclic alternation between nutrient deprivation and plentiful prey while swarming lost developmental proficiency within one year of evolution (46). There may be a substantial number of natural environments in which myxobacterial cooperative traits are partially or fully lost owing to relaxed selection for cooperation (49, 112, 114).

**Cheaters**

In the myxobacteria, cheaters might exploit cooperative production of exoenzymes (1), motility surfactants or signals, developmental signals, or secondary metabolites and socially defective developmental cheaters can be readily generated (Figure 3). Velicer et al. (113, 114) examined the behavior of several *M. xanthus* socially defective mutants, or obligate defectors, that fail to make one or more signals necessary for normal development. Some defectors were isolated from populations that had spontaneously evolved in liquid culture (112, 114), whereas others carried defined mutations in developmental genes. When mixed as a minority with their developmentally proficient ancestor, more than half of these defectors exhibited a cheating phenotype by sporulating more efficiently than the cooperator (Figures 2a, 3). Several *M. xanthus* cheaters exhibit severe (in some cases total) social defects in pure culture associated with extremely large fitness advantages (10- to 100-fold) when rare in mixed groups (Figure 3) (113, 114).
Social Collapse

Selfish behavior can cause the breakdown of cooperation among microbes (25). Indeed, when mere survival requires cooperation, socially defective cheaters can drive populations to outright extinction. Fiegna & Velicer (25) examined the competitive fates of several socially defective \textit{M. xanthus} cheaters over repeated cycles of development and growth in mixes with a socially proficient cooperator (wild type, WT). In these experiments, only spores survived selection imposed at the end of each developmental cycle. Two of these cheaters induced large population crashes during development when they rose to high frequency and thereby decreased group productivity. One of the cheaters (obligate cheater, OC) imposed particularly severe cheating load and caused outright extinction events during these population crashes. In some replicate populations OC alone went extinct, whereas in others the entire population of both cheaters and cooperators died off. These extinctions exemplify evolutionary suicide, in which adaptations that increase the fitness of individuals in the short run drive populations or species to extinction in the long run (86).

What Limits Socially Defective \textit{M. xanthus} Cheaters?

Developmentally proficient strains of \textit{M. xanthus} are common in soils (118, 119), so what forces limit the spread of socially defective cheaters in this species (108)? First, it is likely that social defect mutations allowing cheating on one \textit{M. xanthus} social trait (e.g., development) often have negative pleiotropic effects on some other major component of fitness (e.g., motility and/or predation) (80, 112, 113). Second, cheaters that increase within their group of origin may often fail to successfully migrate into other groups if kin discrimination commonly allows territorial exclusion of nonkin in \textit{M. xanthus} (see below; 121). To the extent that territorial exclusion limits migration, relatedness will be increased and cooperation promoted. Hypothetically, high relatedness among cooperators might also be promoted by use of the A-motility system by cooperators to emigrate from cheater-infected groups and establish new cheater-free colonies (108). High relatedness will allow groups of pure cooperators to contribute more individuals to future generations than groups heavily burdened by cheating load, which can be severe in \textit{M. xanthus} (Figure 3) (25).

Negatively frequency-dependent fitness may prevent cheaters from completely over-taking cooperators within cheater-infected groups. Under laboratory conditions, some \textit{M. xanthus} cheaters lose their fitness advantage over cooperators at high cheater frequencies (Figure 3) (113). If similar relationships occur in the soil, selection will maintain both cooperators and cheaters within social groups. Consistent with this possibility, developmentally defective cheaters were found to oscillate in frequency rank with cooperators over several cycles of development in laboratory experiments (25). Finally, novel cooperative genotypes can evolve that are resistant to exploitation by cheaters (27; P. Manhes & G.J. Velicer, manuscript submitted).

THE RE-EVOLUTION OF COOPERATION

Myxobacteria can rapidly lose their social capabilities due to either relaxed selection for cooperation at the group level or selection favoring socially defective cheaters at the individual level. Such defective genotypes should frequently persist at least transiently in natural populations either due to local selective conditions that favor them or by genetic drift. Must such social degradation be an evolutionary dead end for cooperation within a particular lineage? Or might some mutational pathways allow socially defective genotypes to regain social proficiency and thereafter succeed in environments that favor cooperation? Laboratory experiments have shown that \textit{M. xanthus} has a striking capacity to spontaneously re-evolve cooperative functions from socially defective
Moreover, it can do so by mutations that generate novel genetic interactions rather than by simple reversion of defect mutations.

**A Developmental Phoenix**

Although the strain OC often drove itself and whole populations to extinction in the Fiegna & Velicer (25) study described above, in one case it escaped such a fate by spontaneously re-evolving developmental proficiency through a single mutation (Figure 4a) (27). The new mutant (Phoenix, PX) sporulated more efficiently than the WT cooperator, against which OC had initially competed, both in pure culture and over a wide range of relative frequencies in mixed cultures. Strikingly, PX was also competitively dominant over its immediate parent OC at all mixing frequencies. Thus, evolutionarily restored development by PX is resistant to cheating by OC even though OC can severely exploit its own direct ancestor, WT. The state of being a socially defective cheater thus served as an evolutionary stepping-stone to a new and competitively dominant mode of cooperation.

The PX genome was sequenced and 15 mutations distinguishing PX from WT were found, but only one was unique to PX while the other 14 were already present in OC (Figure 4a) (116). The PX mutation directly confers the PX phenotype in the OC background (27). This mutation occurred in an intergenic region and appears to have disrupted the function of a regulatory small RNA molecule (Y.-T.N. Yu, X. Yuan & G.J. Velicer, manuscript submitted). The PX mutation causes many unique patterns of developmental gene expression (relative to both WT and OC) (52) and mediates a novel mechanistic pathway to developmental proficiency in *M. xanthus*.

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**Figure 4**

Re-evolution of (a) development and (b) social swarming. (a) The lineage from wild type (WT) to the obligate cheater (OC) (see main text) accumulated 14 mutations during 1000 generations of evolution in liquid and lost developmental proficiency (112, 116). OC evolved into the developmentally proficient strain Phoenix (PX) by a single mutation during four cycles of competition with a marked variant of WT (27, 116). Images reprinted with permission from Reference 27. (b) Soft agar colony phenotypes of the WT parent (top position), two sibling ΔpilA mutants (second and fourth positions counterclockwise from top) derived from WT (blue arrows) and their evolved descendants E7 and E8 (third and fifth positions counterclockwise from top, respectively, red arrows). Reprinted with permission from Reference 117.
Re-Evolution of Social Swarming

In addition to re-evolving social development, *M. xanthus* can also spontaneously re-evolve social cell movement from a defective state (117). S-motility was eliminated by deletion of the *pilA* gene [which encodes the pilin subunit of Type IV pili (134)] from the lab strain DK1622 (here WT). Replicate populations derived from Δ*pilA* ancestors underwent successive two-week growth cycles on soft agar, on which S-motility drives effective swarming in WT but A-motility does not (Figure 4b) (45, 98). Population samples farthest from the initial point of inoculation were transferred to fresh plates at the end of each cycle so that selection favored mutants that swarm more rapidly than their ancestors. After 32 cycles of evolution, six populations exhibited moderate increases in swarming rate, but two others, populations E7 and E8, improved much more dramatically (Figure 4b).

The re-evolution of group swarming by lines E7 and E8 was not accomplished by regaining the ability to make pilin, but rather by evolutionary cooption of the A-motility system. Like their Δ*pilA* ancestors, E7 and E8 fail to make Type IV pili. Unlike their ancestors, however, E7 and E8 produce high levels of EPS. In the pili-producing parent of the two Δ*pilA* ancestors (WT), pili synthesis positively regulates EPS production (4, 117), which was effectively eliminated in the ancestral Δ*pilA* mutants. However, the ability to produce EPS independently of pilin production evolved in both E7 and E8. Both evolved EPS production and the A-motility system were integral to the re-evolved swarming phenotypes. Thus, a novel interaction between *M. xanthus* EPS and A-motility evolved to allow effective swarming across a surface type that previously could not be accomplished by cells lacking pili-mediated S-motility. The E7- and E8-evolved swarming phenotypes were also social traits that involve EPS-mediated cell-cell interactions (rather than merely EPS production per se). Mutations accumulated in the E8 lineage that have been identified by whole-genome sequencing will shed light on the molecular mechanisms by which E8 regained a swarming phenotype (G.J. Velicer, unpublished data). The occurrence of evolutionary transitions to novel forms of development and social swarming from socially defective backgrounds in laboratory populations suggest that similar transitions might occur in natural populations.

SOCIAL FRAGMENTATION IN STRUCTURED POPULATIONS

Once stabilized against the threat of socially defective cheaters, microbial cooperation will thrive at least among clone-mates, but distinct lineages of socially proficient genotypes will diverge and compete. Such competition between genotypes that cooperate with their own kind may be mediated by direct behavioral interactions either within heterogeneous social groups or between homogeneous but neighboring groups. Fiegna & Velicer (26) examined whether, and to what degree, developmentally proficient lineages of *M. xanthus* from distant locations have diverged into socially incompatible genotypes. Cells of nine strains isolated from around the globe were mixed at a 1:1 ratio in all possible pair-wise combinations (36 total) at the onset of starvation, and the effect of genotype mixing on spore production by each paired strain was quantified.

In almost all pairings, mixing of strains greatly reduced spore production by one or both competitors (as exemplified in Figure 2c). Intriguingly, the winning strains in several pairings exploited their competitors for an absolute gain in survival rate by producing more spores in their competitors’ presence than when alone in clonal groups (Figure 2c, top outcome of chimerism).

The diversification of *M. xanthus* into many socially antagonistic genotypes prompts several questions, including, At what scales of spatial distance and genomic divergence do social incompatibilities emerge? What are the relative contributions of natural selection and genetic drift to social divergence? What molecular mechanisms underlie these antagonisms? Below we review current knowledge of how
MLST: multi-locus sequence typing

genetic and phenotypic diversity is spatially structured in natural *M. xanthus* populations.

**M. xanthus** Diversity at the Centimeter Scale

The spatial distribution of intraspecific bacterial diversity and the ecological and evolutionary forces that maintain it are only beginning to be understood (68, 82). Centimeter-scale patches of soil are large enough to contain many distinct *M. xanthus* genotypes but small enough that genotypes coexisting at this scale are likely to encounter one another through active motility and passive dispersal. One hundred soil samples were collected from a 16 × 16 cm patch of soil in Tübingen, Germany, and individual *M. xanthus* clones could be isolated from 78 of these samples (118). Among these isolates, 21 genotypes were distinguished on the basis of a three-gene (*csgA, fibA*, and *pilA*) multi-locus sequence typing (MLST) (67) concatemer. Accumulation curves indicated that most of the variation present in the sampled plot at the concatemer loci was represented among the sampled clones. Genotypes were not significantly clustered in the sampling grid, implying that clonal patchiness occurs below the centimeter scale (118).

In addition to being genetically diverse, this set of centimeter-scale isolates was also found to harbor extensive phenotypic diversity in several traits, including swarming rate and morphology (120), rate of development, fruiting body morphology, spore production (59), secondary metabolite production (60), and prey range (A. Morgan, C. MacLean & G.J. Velicer, manuscript submitted). The extent of phenotypic variation observed in the laboratory among these isolates strongly suggests that such traits also vary functionally in natural soil habitats.

**Spatial Population Structure**

The field of microbial biogeography is growing rapidly but remains in its infancy. One long-standing problem that remains unexplored for most free-living microbes is the degree to which they disperse across both small and large scales (77). Myxobacteria cells may be particularly amenable to extensive dispersal as durable spores and therefore offer a conservative test case for the presence of spatial structure and limited dispersal in free-living bacteria. Genetic diversity at five MLST loci was measured among 145 clones of *M. xanthus* isolated in a nested sampling design across nine spatial scales ranging from centimeters to thousands of kilometers (119). In addition, the degree of genetic differentiation among meter-scale populations separated by hundreds to tens of thousands of kilometers was quantified. *Myxococcus* populations were found to be highly structured at large spatial scales. Both total diversity among the hierarchically nested clones (Figure 5a) and the degree of genetic differentiation between local meter-scale populations (Figure 5b) were found to increase with distance across large spatial scales (≥ hundreds of km). Differential patterns of genetic drift resulting from the spatial isolation of allopatric populations (i.e., limited dispersal) appear to be largely responsible for the observed patterns of population differentiation (119).

**Scales of Social Divergence**

At what spatial and genetic scales do social incompatibilities such as the developmental antagonisms found among global *M. xanthus* isolates (26) first evolve? Because dispersal is limited, incompatibilities among global-scale isolates may result from the overall divergence of allopatric populations due to genetic drift and/or local adaptation. Alternatively, incompatibilities might first evolve at small spatial scales among highly similar sympatric lineages. To distinguish between these hypotheses, Vos & Velicer (121) compared competitive interactions in chimeric developmental cultures among genetically similar clones isolated at the centimeter scale to the interactions among global isolates examined by Fiegna & Velicer (26).
Figure 5

(a) Chao1 nucleotide richness estimates based on sample sets representing a wide range of spatial scales (centimeter to global). Each data point represents the estimated number of polymorphisms in a multi-locus sequence typing (MLST) concatemer in a population at a given scale. Adapted with permission from Reference 121. (b) $F_{ST}$ values among 10 globally distributed meter-scale populations plotted against distance between populations. $F_{ST}$ values represent the degree of genetic differentiation between paired populations. Significantly differentiated population pairs are indicated by triangles; nonsignificantly differentiated population pairs are indicated by squares. (Panels a and b reprinted with permission from Reference 119). (c) Codevelopment interactions among three centimeter-scale isolates that share identical MLST concatemer sequences (121). The effect of pair-wise mixing on the (log-transformed) sporulation efficiency of an individual competitor is termed the one-way mixing effect ($C_{i(j)}$). Open bars show the mixing effect of the first clone in each pair and gray bars show the mixing effect of the second clone. Error bars represent 95% confidence intervals. (d) Kin discrimination between swarms of clones sharing identical MLST genotypes. A clear line of demarcation is evident between swarms of isolates A23 and A47. The control treatments show two swarms of the same clone that have merged into a continuous swarm with no line of demarcation. (Panels c and d reprinted with permission from Reference 121.)

Nine clones from the centimeter-scale sample set described above were mixed at the onset of development in all possible pair-wise combinations. The average pair-wise genetic distance among these local isolates was almost half of that among the global isolates, and three of the local isolates share the same MLST genotype (121). Just as among the global isolates, the average effect of pair-wise mixing on the spore production of individual local competitors was found to be strongly negative. Intense antagonisms were observed even among the three isolates sharing identical MLST loci, including interactions in which chimerism enhanced the absolute performance of the winner (Figure 5c). Intriguingly, the average negative
effect of mixing on individual spore production was smaller among the local strains than among global isolates. Additionally, the average total spore production of mixed groups was significantly reduced relative to clonal groups among the global strain competitions but not among the local strains. Overall, these results show that strong social incompatibilities evolve within local *M. xanthus* populations among genetically similar neighbors, but also suggest that such incompatibilities may be augmented by additional genetic divergence across allopatric populations.

### Kin Discrimination Among Swarms

Because *M. xanthus* cells tend to adhere to one another during growth on surfaces, many encounters between *M. xanthus* genotypes in the soil are likely to occur at the interfaces of clonal (or nearly clonal) groups. To investigate between-group interactions during vegetative growth, the ability of paired genotypes from the 78-cm-scale Tübingen isolates (118) to merge into single continuous swarms was examined. Swarming incompatibility was pervasive. While distinct swarms of the same clone freely merge, most paired isolates failed to do so, including when they shared identical MLST genotypes (Figure 5d).

The swarming incompatibilities and pervasive developmental fitness asymmetries in heterogeneous populations observed among genetically similar centimeter-scale isolates suggest that kin discrimination and social antagonism can evolve rapidly (relative to the genome as a whole) within local populations. The ability of clonal swarms to prevent merger with distinct genotypes in the wild would serve as a general strategy of xenophobia (108) to prevent territorial invasion by cheaters and thereby promote high relatedness. Even a small number of polymorphisms can generate kin discrimination in some microbes. For example, some single mutations prevent distinct swarms of the bacterium *Proteus mirabilis* from merging (35). The molecular and evolutionary mechanisms that generate and maintain kin discrimination in microbes (35, 84, 102, 121) are of great interest for future research.

### PERSPECTIVES AND PROSPECTS

The young field of sociomicrobiology is attracting researchers with diverse backgrounds in microbiology, ecology, and evolutionary biology to pursue questions that require interdisciplinary integration. Myxobacterial social evolution poses a particularly fascinating set of problems. These include basic questions about how cooperation is stabilized that apply to all cooperative microbes, as well as more specific questions regarding the coevolution of multiple social traits, coevolution between social predators and their prey, re-evolution of lost social traits by novel genetic pathways, and evolutionary increases in the genetic, molecular, behavioral, and morphological complexity of social traits. *Myxococcus xanthus* is a powerful model system for addressing many such questions.

Our understanding of how biological systems evolve is being increasingly applied to medical, agricultural, conservation, and environmental problems (10). Many myxobacteria species can prey upon a variety of pathogenic microbes in a social manner and some can reduce pathogen damage to crops when released on infected hosts (9). Natural or experimentally evolved myxobacterial predators (46) and the compounds they produce might be utilized as effective biocontrol agents (3, 115).

### SUMMARY POINTS

1. Social cooperation and conflict are pervasive among microbes.
2. The character and benefits of cooperative traits can be challenging to identify and quantify in natural populations of microbes.
3. Socially defective cheaters can readily evolve in many social microbes, including *M. xanthus*. Cheating in nature is likely to be limited by a complex combination of genetic, behavioral, and population-structure mechanisms.

4. Myxobacterial cooperation can spontaneously re-evolve from some socially defective genotypes.

5. Genetic variation in natural populations of *Myxococcus* is spatially structured owing, at least in part, to limited dispersal.

6. Social incompatibilities evolve among genetically highly similar individuals living in sympathy.

**FUTURE ISSUES**

1. What specific benefits are gained by myxobacteria through cooperative behavior in nature?

2. What range of mutations allow successful cheating on *M. xanthus* social traits? Can social motility and predation be exploited by cheaters? To what degree have the genetic networks that underlie myxobacterial social traits evolved to be robust against potential cheating mutations?

3. At what frequencies are socially defective cheaters maintained in natural populations and what limits them?

4. How readily can a wide range of socially defective genotypes indirectly re-evolve proficiency at cooperation?

5. How frequently are myxobacterial social groups chimeric in natural populations? Do some environmental conditions favor spontaneous merger of distinct genotypes into heterogeneous social groups, whereas others do not?

6. What mutations and molecular and evolutionary mechanisms drive the evolution of social incompatibilities?

7. What are the evolutionary, molecular, and behavioral mechanisms responsible for the diversity in fruiting body morphologies observed across myxobacterial species?

**DISCLOSURE STATEMENT**

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