



Social strife in the microbial world

Gregory J. Velicer

Department of Evolutionary Biology, Max-Planck Institute for Developmental Biology, Spemannstrasse 37, 72076 Tübingen, Germany

The natural life histories of microorganisms involve complex social interactions that occur both within and between species and can be either competitive or cooperative. Flourishing research on biofilms and quorum-sensing systems has heightened interest in how and when microbes cooperate to obtain group-specific fitness advantages. However, 'cheaters' can disrupt cooperative systems by unfairly procuring an excessive share of group-generated resources while making disproportionately small contributions. Evolutionary theory predicts that such 'selfish' genotypes should readily invade cooperative populations in the absence of mechanisms to exclude them. Recent studies reviewed here have revealed selfish strategies that undermine cooperative behaviour across a variety of microbial systems, including eukaryotic slime moulds, bacteria and viruses. Further empirical understanding of microbial cheating will provide insight into the limits on cooperative behaviours and the hurdles to transitions from unicellularity to multicellularity.

The idea that cooperative behaviour between microbial organisms is commonplace has gained widespread acceptance in recent years [1–3]. Sophisticated behaviours, such as fruiting body construction in the myxobacteria and eukaryotic slime moulds, have long been recognized as cooperative interactions [4]. More recently, research on microbial biofilms and quorum-sensing systems has helped drive the understanding that most microbes live out their life histories in social contexts that involve complex webs of both cooperative and competitive interactions [5–7]. Although hypotheses about the precise nature of putative COOPERATION (see Glossary) among microbes should be carefully tested [8], numerous microbial behaviours are difficult to account for without reference to some type of cooperation or even ALTRUISM.

Crespi [3] noted that major categories of social cooperation among higher eukaryotes have analogs in the microbial world (see Table 1 of Ref. [3]). Bacterial biofilms can be likened to protective domiciles, such as nests or hives. Nitrogen-fixing cells of *Rhizobium* and cyanobacteria filaments are specialized food providers analogous to the foraging classes of social insects. Intercellular chemical signals in bacteria correspond to pheromones in social insects and animals. Cooperative feeding in the myxobacteria has been compared with pack hunting in wolves and lions [9]. Strikingly, some microbial behaviours have been interpreted as extreme forms of

altruism, such as the cases of stalk-cell death in *Dictyostelium* fruiting bodies [10], autolysis during *Myxococcus* development [11], self-destructive toxin production by bacteria [12] and some instances of *Escherichia coli* phage exclusion [13].

For microbes, as well as for higher eukaryotes, different types of cooperation can be distinguished (Box 1). Most simply, mere restraint from conflict over a pre-existing PUBLIC GOOD (i.e. a resource not generated by the group that uses it) can be described as a cooperative behavioural strategy [14]. More frequently, cooperation is conceived positively as any behaviour that is costly to the individual but that helps to generate a public good that otherwise would not exist or would be inaccessible. This article focuses on cooperation in this positive sense and, within this broad definition, distinguishes between three types of cooperation: minimal, density-enhanced and group-limited cooperation (Box 1). Cooperative behaviour can increase the evolutionary fitness of individuals in cooperative groups when individual survival or reproductive rate is limited by some COLLECTIVE ACTION of the group [15,16]. Cooperation is often thought to evolve most readily within groups of highly related individuals [17], although interesting fitness benefits have been associated with genetic diversity in the societies of some insects [18,19].

Cheating

All types of cooperation that involve the costly generation or acquisition of a common pool of resources are in principle subject to CHEATING. DEFECTION from cooperative strategies

Glossary

Altruism: behaviour that confers fitness benefits on other individuals while costing its performer a net reduction in evolutionary fitness.

Cheating: obtaining benefits from a collectively produced public good (see below) that are disproportionately large relative to a cheater's own contribution to that good.

Cheating load: the degree to which obligately defecting cheaters decrease the group-level benefits of cooperation in chimeric social groups.

Collective action: the combined effect of individual behaviours within a group.

Cooperation: proportional contribution by individuals to a collectively produced public good. (See Box 1 for distinctions between types of cooperation.)

Defection: disproportionately small contribution by individuals to a collectively produced public good. Biologically, defection does not necessarily entail cheating (i.e. gaining an advantage from defection). Some mechanisms of defection might not enhance the relative ability of defectors to exploit a relevant public good.

Public good: any fitness-enhancing resource that is accessible to multiple individuals within a local group. A pre-existing public good (such as rainwater) originates independently of the group that benefits from it. Alternatively, a collectively produced public good (such as an intercellular signal) is generated by members of the group that utilize it.

Corresponding author: Gregory J. Velicer (gregory.velicer@tuebingen.mpg.de).

Table 1. Cooperative traits and their benefits

Cooperative trait	Possible density-enhanced or group-limited benefits	Refs
Fruiting body construction (e.g. <i>Myxococcus</i> , <i>Dictyostelium</i>)	Dispersion, protection from predation and caustic compounds, 'wolf-pack' feeding after germination	[53]
Stationary-phase growth cessation	Increased survival rate	[36]
Viral gene expression during co-infection	Increased host-exploitation efficiency	[32]
Mutualistic nitrogen fixation and other inter-species mutualisms (e.g. rhizobia-legume symbioses)	Access to plant nutrients	[52]
Siderophore production	Enhanced growth under iron limitation	[35]
Biofilm formation	Niche exploitation, protection from host defences or caustic environmental compounds	[6,66,67]
Interference competition, allelopathy (e.g. <i>Escherichia coli</i> colicin production)	Availability of substrates not consumed by inhibited competitors	[12,68]
Extracellular metabolism of impermeable growth substrates (e.g. prey organisms, cellulose, etc.)	Increased local concentration of extracellular enzymes	[49,69]
Quorum-sensing signal production	Delay of costly gene expression until quorum-limited benefits can be realized	[5,15,50]

of social interaction allows 'cheaters' to gain the advantages of cooperation without bearing its costs. The potential for cheating is widespread, since interactions between individual organisms are not the only contexts in which 'games' with cooperative and selfish strategies are played out. Many levels of biological hierarchy can be viewed as societies of cooperating entities [20,21]. Most genes within a chromosome cooperate to generate a protective domicile (the cell) and to reproduce their entire society in a 'fair' manner. But some genetic elements, such as transposable elements and meiotic drive chromosomes [22], are able to escape the fair replication constraints imposed on most genes and become disproportionately represented in subsequent generations. Similarly, selfish cancer cells in the soma of multicellular organisms escape growth controls and excessively proliferate in a manner that undermines the cooperative task of maintaining a healthy organism that can reproduce through a sequestered germ line [23]. Cheating even occurs in eusocial insect colonies, which are often viewed as archetypes of social cooperation, but in fact are susceptible to many forms of social conflict [24]. All of these societies are under threat from selfish individuals, because natural selection can act at multiple levels [25] and cheaters could gain an individual advantage to the detriment of the cooperative group that they exploit.

The evolutionary outcomes of cooperative versus selfish social strategies can be formally analysed using evolutionary game theory (Box 2). Regardless of the level of biological complexity, game theory quantifies the fitness payoffs of cooperative and selfish behavioural strategies in particular social systems and allows the long-term competitive fates of cooperative and selfish individuals to be predicted. As long as the assumptions (i.e. the rules of the game) are met, then game theory allows predictions to be generated.

Cheating: full-time or part-time?

Cheating can be accomplished by either obligate or facultative defection from cooperation. Obligate defectors are inherently deficient at the very cooperative trait that is being exploited, and their presence could have severe effects on the group as a whole [26]. Thus, individuals within pure defector groups are less fit than those in pure cooperator groups when the relevant cooperative trait is

important for overall fitness. To succeed evolutionarily, obligate defectors require the proximate presence of their exploited social host. For example, some species of social insects are obligate defectors that are unable to produce their own worker caste and parasitically rely on workers produced by queens of another species to raise their brood [27,28]. Within a mixed group of cheaters and cooperators, cheaters that obligately defect will inherently decrease the average share of a pooled benefit as a function of defector frequency. In doing so, they reduce fitness by imposing a CHEATING LOAD on groups of exploited cooperators.

Facultative defection is more sophisticated and has been observed in some social insects [29,30]. It requires the ability to: (1) distinguish between close kin and non-kin; (2) cooperate with close kin; and (3) modify behaviour to be specifically exploitative towards non-kin. (Note that behavioural ecologists do not normally consider the reception of altruistic help from very close kin to be a form of defection.) Fully facultative defection has the advantage of not causing decreases in mean fitness within a social group as facultative defectors increase in frequency. Because of its more complex requirements, facultative defection should generally be more difficult to evolve among microbes than obligate defection, which might originate from a simple mutation that results in non-contribution to an important public good.

Studies of social strife in microbes

Benefits of group living are tempered by various forms of potential conflict between close neighbours [31], including selfish individuals cheating on cooperative ones. Several recent studies document evolutionarily advantageous defection from cooperative strategies in four very different microbes (Fig. 1). Social conflict in co-infecting RNA viruses, stationary phase *E. coli* cultures, and developing populations of the social bacterium *Myxococcus xanthus* all involve obligate defection, but some selfish strains of the social amoeba *Dictyostelium discoideum* might defect facultatively. Although these systems differ dramatically, they all share at least one commonality: survival or reproductive success is determined by the collective action of social groups. This common thread provides the basis for cheating and social conflict to evolve. It also provides clues about the generality of cheating among microbes and the mechanisms by which it could occur.

Box 1. Three types of cooperation

Cooperation has been defined generally as the investment of 'resources in a common interest shared by other group members' [58]. The crucial feature of this definition is the adoption of a costly behavioural strategy that can benefit neighbours, regardless of whether or not they exhibit the same strategy. Neighbours that defect from contributing to the common pool can nonetheless benefit from it. Within this general definition, three distinct types of cooperation are distinguished here: minimal, density-enhanced and group-limited cooperation.

Minimal cooperation is simply restraint from 'unfairly' sequestering a benefit generated by group members when the magnitude or mere existence of that benefit is not density dependent. Thus, a minimally cooperative trait is equally beneficial in isolation or in pure groups of cooperators. For example, the secretion of any diffusible substance (such as a siderophore, cellulolytic enzyme or anti-competitor toxin) that acquires, releases, or protects previously inaccessible or threatened resources might confer a net benefit to isolated cells without providing a greater benefit to its producers when they are clustered in groups. However, in groups, production of such a compound can benefit any neighbour close enough to partake of the resulting resource pool, even if those neighbours did not contribute to the pool. In mixed groups of contributors and non-contributors, there is a relative cost to contribution, which is therefore described as cooperative. Minimal cooperation is maintained solely by individual-level selection. Selection should therefore favour isolated living rather than social life histories because proximate cooperators do not enhance each other's fitness in group living, whereas any neighbouring defectors decrease the fitness of all local cooperators.

More commonly, cooperation is conceived as positively synergistic, where groups of individuals gain some benefit together that they could not obtain in isolation. Density-enhanced cooperation occurs if a trait that is beneficial in isolation provides an even greater benefit to its bearers in a high-density group of cooperators than at low density. Thus, the magnitude of a benefit, but not its mere existence, is density dependent. For example, isolated cells of *Myxococcus xanthus* are capable of killing prey and feeding on their remains [59]. However, the efficiency of *M. xanthus* predation is thought to be positively density dependent [49]. If this is the case, individuals in high-density groups will reproduce at a faster rate than isolated individuals in otherwise identical prey environments. When a density-enhanced cooperative trait is a major component of fitness, natural selection should favour cells that tend to cluster in high-density groups.

Group-limited cooperation is a more complex type of synergistic cooperation and occurs when a behaviour only produces a fitness benefit in sufficiently large and dense groups of cooperators. Such a trait is disadvantageous if expressed at low density. Some instances of intercellular signalling could constitute group-limited cooperation if secreting a signal provides no selective advantage to isolated individuals [8]. Because natural selection at the level of individual fitness effects is usually stronger than selection on shared benefits, the evolution of group-limited cooperation (as well as the related phenomenon of altruism) requires a stricter set of conditions than does the evolution of less-complex types of cooperation.

Imprisoned viruses

Working near the bottom tier of biological complexity, Turner and Chao asked whether social conflict might be at work in co-infecting populations of the RNA virus $\phi 6$ [32] (Fig. 1a). After host cell infection, viruses manufacture diffusible gene products used for genome replication and virion construction. When multiple viruses infect the same host cell, this pool of gene products is accessible to all replicating genomes. In the case of multiple infections by genotypically distinct viruses, some genotypes might make contributions to the public pool of gene products

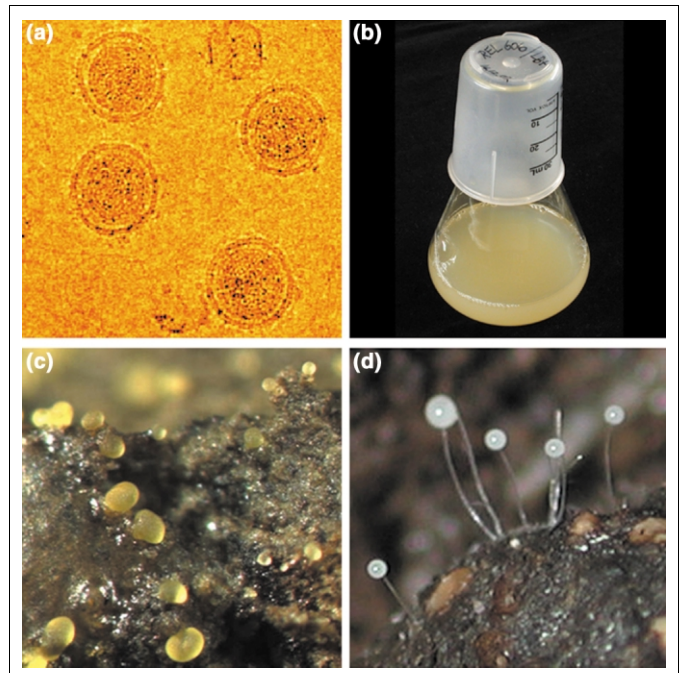


Fig. 1. Four microbial social systems subject to defection. (a) RNA virus $\phi 6$ (photo by Dr Sarah Butcher, University of Helsinki). (b) A stationary-phase culture of *Escherichia coli*. (c) *Myxococcus* fruiting bodies on soil from Hainich National Park, Germany (photo by Michiel Vos, Max-Planck Institute for Developmental Biology). (d) *Dictyostelium* fruiting bodies on deer scat at the Mountain Lake Biological Station, Virginia (photo by Thomas Platt, Rice University).

that are proportional to their frequency in the population. Such a strategy is cooperative in the context of game theory, which analyses the fitness implications of contributing (or not) to a fitness-limiting public good generated by collective action. Alternatively, selfish genotypes might contribute less than their 'fair share' to the pool, but sequester more than their share. When the quantity of viral gene products is growth limiting, reproduction in host cells infected primarily by selfish defectors should be less efficient than in cells with mostly cooperative types, which take portions from the shared pool that are proportional to (or less than) their own contributions.

Turner and Chao subjected multiple lineages of $\phi 6$ derived from a common ancestor to 50 sequential cycles of bacterial host infection under conditions of either low or high multiplicity of infection (MOI) (MOI of 0.002 and 5, respectively) [33]. Selection at high MOI surprisingly led to the eventual evolution of decreasing fitness over time. This was interpreted as resulting from the evolution of interference competition between co-infecting genotypes, with some defectors sequestering more than their share of intracellular resources. The mixing of variable genotypes in the high MOI treatment provided a within-cell selective context that favoured the rapid evolution of genetic conflict. Alternatively, at the low MOI treatment, cells were almost always infected by only a single genotype. Under these conditions, selection between genotypes that varied primarily between host cells (rather than within them) served to increase host exploitation by single genotypes rather than within-host conflict. The fitness of low-MOI lines continued to increase throughout the entire selection experiment.

Box 2. Evolutionary game theory for microbes

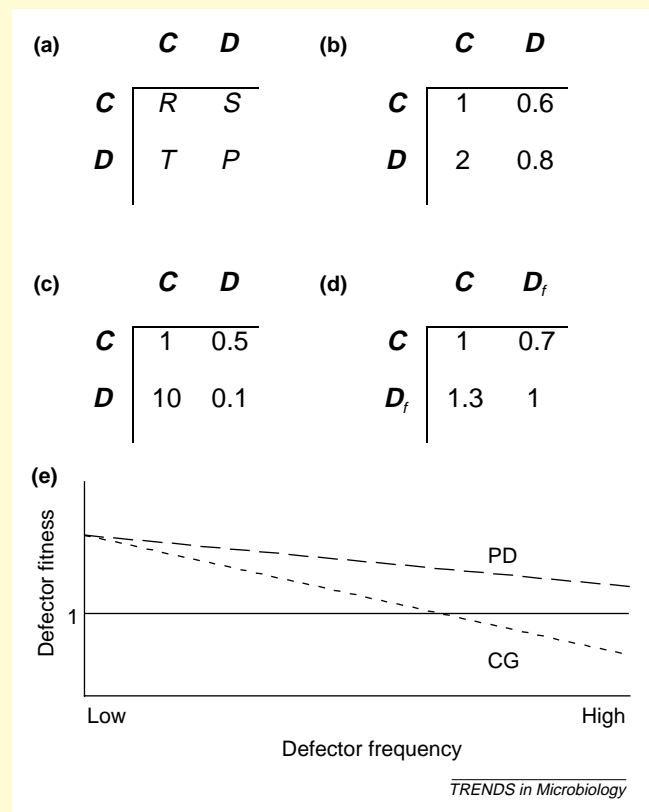
Game theory analyses gains and losses resulting from distinct strategies of interacting players that compete for some benefit under defined constraints (Fig. 1). Decisions inherent in gambling, military, business and political strategizing and other human activities all fall under the purview of game theory analysis. In 1973, Maynard Smith and Price showed how game theory illuminates the evolutionary implications of various animal behaviour strategies [60]. Since that time, game theory has been extensively used to model conditions allowing the evolution of cooperation and altruism [61–63]. Recently, the applicability of game theory to microbial social interactions has been increasingly recognized [14,32,36,64]. Though games are often conceived as having just two players, the players can also be large populations of microbes within which individuals exhibit distinct social strategies.

The prisoner's dilemma [14,63] is perhaps the best-known application of game theory in biology and is often used to illustrate the perennial problem of how cooperation can be maintained in the presence of non-cooperative, or 'defector' genotypes. The prisoner's dilemma has two possible strategies, to cooperate (*C*) with other players or defect (*D*), and stipulates that defection is advantageous at all defector frequencies (Fig. 1b,e). If all players cooperate, their payoff (*R*, reward for cooperation) will be higher than if all defect (*P*, punishment for mutual defection). But if a majority cooperates while a minority defects, the defectors gain a fitness payoff (*T*, temptation to defect) that is greater than *R*. Cooperators receive a 'sucker's payoff' (*S*) that is lower than *P*. Thus, $T > R > P > S$. Defection guarantees that other players will have either an equivalent (*P*) or worse (*S*) payoff than one's own. Defecting in a prisoner's dilemma context guarantees evolutionary success because

defection either ties or wins relative to other players, depending on whether they defect or cooperate, respectively. Inversely, cooperators must either tie or lose in the calculus of relative fitness, making cooperation a losing strategy in the long run. (For a more extensive treatment of prisoner's dilemma games, see Ref. [63].) A selfish strain of the RNA virus $\phi 6$ [32] (Fig. 1b) and *Escherichia coli* stationary phase GASP mutants [36] have both been shown to exhibit the prisoner's dilemma.

In the so-called chicken game [65], the rank order of *P* and *S* are reversed ($T > R > S > P$) so that, when defectors become very frequent, individuals are better off cooperating than defecting as well (Fig. 1c,e). Some *Myxococcus xanthus* cheater strains show a chicken game relationship to their cooperative counterparts during social development [44] (Fig. 1c). In games of chicken, the frequency-dependent reversal of fitness superiority between cheaters and cooperators promotes the maintenance of genetic variation in mixed populations [26].

Fig. 1. Game theory fitness matrices and frequency-dependent defector fitness (see Box 2). (a) Qualitative payoffs from particular interactions between cooperators (*C*) and defectors (*D*) [14]. For interactions in microbial populations with many individuals, consider that each payoff applies to a small minority of individuals of the type listed on the left when surrounded by a large majority of individuals of the type listed on the top (also for Figs 1b–d). See Box 2 text for definitions of *P*, *R*, *S*, and *T*. In Figs 1b–d, the payoff for cooperators interacting with only cooperators (upper-left in each matrix) is set at one [64]. (b) Fitness matrix conforming to the prisoner's dilemma, where $T > R > P > S$. These values approximately correspond to empirical values calculated for cooperating and defecting viral types in Turner and Chao [32]. (Figs 1b and c are modified from Ref. [64].) (c) Fitness matrix conforming to the chicken game, where $T > R > S > P$. These values reflect approximate fitness relationships between wild-type and cheating strains of *Myxococcus xanthus* [44,64]. (d) Facultative defection. Here, a selfish genotype (*D_f*) only defects in the presence of an unrelated cooperative type (*C*), but facultatively switches to a cooperative phenotype when interacting only with its own type. Thus, facultative defectors have an advantage at all frequencies in mixes with unrelated cooperators, but there is no punishment of defection for pure groups of *D_f* (i.e. lower-right payoff = 1). Some strains of *Dictyostelium discoideum* might be capable of facultative defection [45]. (e) Hypothetical defector fitness (relative to cooperators) as a function of defector frequency in mixed groups for the prisoner's dilemma (PD) and chicken game (CG). Reproduced, with permission, from Ref. [64].



TRENDS in Microbiology

One defector clone ($\phi H2$) isolated from a high-MOI lineage [33] was shown to exhibit fitness relationships to the ancestor that conform to a classic theoretical game called the 'prisoner's dilemma' (Box 2) [32]. When $\phi H2$ and $\phi 6$ were first mixed at various ratios and then allowed to co-infect host cells at high MOI, $\phi H2$ showed a negatively frequency-dependent fitness advantage over $\phi 6$ at all experimental and extrapolated mixing ratios. Thus, defection was always advantageous when cooperative genotypes were present (i.e. $T > R$; Box 2), but was most beneficial when defectors were rare and least advantageous when cooperators were rare. When $\phi H2$ and $\phi 6$ were first allowed to adsorb to separate host populations at

high MOI and then mixed (resulting in only pure co-infections), $\phi H2$ had a lower fitness than $\phi 6$ ($R > P$; Box 2). Because $\phi 6$ fares worse than $\phi H2$ in co-infections even when $\phi H2$ is at very high frequency, the 'sucker's payoff' for cooperating with defectors is worse than the punishment for universal defection ($P > S$; Box 2) and the prisoner's dilemma conditions are satisfied.

The potential for genetic conflict between co-infecting viruses exemplified in the $\phi 6$ system has implications for the evolution of virulence levels under conditions when multiple infections are common. Contrary to previous theoretical expectations, recent studies suggest that such conflict might often result in the evolution of reduced

virulence owing to the appearance of selfish individuals that prevent the collective action of co-infecting pathogens from maximally exploiting their host [16,34,35].

No rest for the weary

At first glance, *E. coli* growing in liquid culture might appear to be the epitome of microbial individualism (Fig. 1b). However, Vulic and Kolter [36] studied defection from a behaviour exhibited by stationary-phase populations of *E. coli* strain K12 that can be described as cooperative. The 'wild-type' version of this strain ceases population growth before complete depletion of growth substrate in response to several extracellular and intracellular signals. This program of growth cessation results in the transition of cells into a state of reduced metabolic activity (here termed SPR for 'stationary phase rest') that allows prolonged survival under scarce resource conditions. SPR cells slowly utilize residual nutrients to maintain their resting state. Alternatively, GASP mutants (for 'growth advantage in stationary phase') fail to respond to the signal cascade leading to SPR [36]. Instead, they continue a strategy of scavenging nutrients and dividing. In pure cultures, the GASP strategy results in higher death rates (upon resource depletion) than wild-type cells in the resting state [36].

Entry into SPR constitutes group-limited cooperation (Box 1) in liquid culture because the entire population is dependent on the collective action of enough individuals producing sufficient quantities of extracellular signals to trigger the survival-enhancing transition to SPR. Because GASP mutants continue growth after the parental strain has entered SPR, they have a short-term fitness advantage as a minority in mixed cultures. Exploitation of SPR cells that conserve residual resources constitutes cheating by a strategy of obligate defection because the GASP mutants suffer a cost in absolute survival rate (relative to SPR cells) when surrounded only by fellow defectors. To demonstrate cheating, it was shown that GASP defectors appear to have a higher relative fitness than SPR cooperators at all relative frequencies in mixed cultures, with the magnitude of this advantage decreasing with increasing GASP frequency. This set of fitness relationships also conforms to the prisoner's dilemma (Box 2).

Developmental defection

The prokaryotic myxobacteria and eukaryotic social amoebae are primarily soil-dwelling predators that undergo cooperative social development under conditions of nutrient deprivation. Upon depleting proximate sources of amino acids, populations of the bacterium *M. xanthus* migrate towards high-density focal points and cluster into aggregates of ~100 000 rod-shaped individuals. These aggregates transform into mature fruiting structures where a minority of cells differentiate into stress-resistant spores (Fig. 1c) [37]. Similarly, starving populations of the social amoeba *D. discoideum* aggregate into mobile slugs that are spatially heterogeneous with respect to cell fate. Upon extended starvation, slugs transform into stationary fruiting structures that consist of a round, spore-bearing sorus at the top of a long, thin stalk (Fig. 1d) [10].

A notable feature of both *Myxococcus* and *Dictyostelium* development is that distinct portions of aggregated populations have dramatically different fates within the fruiting body. In *M. xanthus*, many cells within fruiting body populations (20–90%, depending on experimental conditions) undergo autolysis during development [11,38,39]. Although various possible developmental functions of autolysis have been proposed, its precise role in *M. xanthus* development remains unresolved [37,40,41]. In *D. discoideum*, ~20% of aggregating populations die while contributing to formation of the stalk and basal disk [10]. Thus, in both cases, multicellular development represents an evolutionary bottleneck through which only a portion of starving populations can pass. Any group-limited fitness benefits conferred by fruiting body formation (which could include enhanced dispersal, protection from predators or caustic compounds and growth rate advantages upon germination) thus come at the cost of death for some individuals.

In mixed populations, such a bottleneck encourages conflict between different genotypes over their relative representation among the limited number of spore slots available. Genotypes able to minimize their likelihood of autolysis (in *Myxococcus*) or stalk construction (in *Dictyostelium*) will have a selective advantage at the individual level within fruiting bodies. Alternatively, simply withholding the costly production of a necessary developmental signal might also confer an anti-social sporulation advantage. In both *M. xanthus* and *D. discoideum*, distinct genotypes can undergo development together as a mixed population (with counteracting costs and benefits of chimeric mixing in *D. discoideum* [42]) and selfish genotypes that are disproportionately represented in spore populations have been identified [43–46].

Velicer *et al.* [44,46] screened several developmentally defective genotypes of *M. xanthus* for frequency-dependent fitness relationships to the developmentally proficient wild-type that would constitute cheating. All eleven genotypes in these two studies were partially or completely defective at spore production (relative to the wild-type) during starvation as genotypically pure cultures. Being unable to cooperate fully with others of the same genotype, they are obligate defectors. However, when mixed as a 1% minority with the wild-type, six of these genotypes cheated during development by producing spores more efficiently than the wild-type. For at least two of these cheaters, their fitness superiority in mixed development is frequency dependent. At high initial cheater frequencies, the relative fitness rank reverses and the wild-type sporulates more efficiently than the cheater. This scenario is an instance of the chicken game in game theory parlance (Box 2). Because both cooperation and defection are advantageous when rare, the chicken game predicts that cooperators and cheaters should be maintained in a balanced polymorphism over sequential cycles of development with oscillating relative frequencies of the two types [26].

Intriguingly, Strassmann *et al.* [45] appear to have identified instances of cheating during *Dictyostelium* fruiting body development that involve facultative defection. In 12 pair-wise mixes of distinct *D. discoideum* natural isolates (1:1 mixing ratio), there were six cases

where one clone was disproportionately over-represented in the pre-spore region (where cells are fated for sporulation) of developing slugs relative to the pre-stalk region, where cells are fated for death by contributing to the fruiting body stalk. One possible means by which some strains could be over-represented among pre-spores in mixtures is a 'fixed-allocation' strategy, where one strain has an unusually high spore:stalk ratio that does not vary between pure and mixed population conditions. However, for at least some selfish strains, evidence suggested that they have normal stalk sizes and a standard spore:stalk allocation ratio (80:20) in pure culture. This would imply that these selfish clones are fully cooperative towards their own close kin, but facultatively defect from cooperation in mixtures with distinct genotypes. Future research might reveal how such facultative cheats distinguish kin from non-kin, and modify their behaviour in response to the relational composition of close neighbours.

Cheats on the rise

The microbial world is replete with costly public goods that could be subject to exploitation (Table 1). Three unfolding studies appear to expand the range of known cheating contexts beyond that of the examples described above. *Saccharomyces cerevisiae* breaks down some growth substrates in the public commons by secreting various hydrolytic enzymes [47]. A strain that defects from producing one such enzyme (and thus does not grow well alone when the enzyme's substrate is limiting) might have a growth-rate advantage over cooperative enzyme producers in mixed groups (D. Greig and M. Travisano, unpublished). Cooperative genotypes of the bacterium *Pseudomonas aeruginosa* secrete siderophore molecules into the public commons to scavenge iron for growth. A spontaneous mutant has been identified that defects from siderophore contribution and therefore grows poorly in pure culture during iron-limited growth. However, in mixed culture with its cooperative parental strain, the defector can have a fitness advantage under iron-limiting conditions due to siderophore exploitation. When iron is not growth limiting, the defector and cooperator genotypes do not appear to differ in their growth rates (A. Buckling, unpublished). On a more structural note, some strains of the common bacterium *Pseudomonas fluorescens* cooperate (at an individual cost) to construct biofilm mats from a secreted matrix of cellulose-like polymer (CLP) [48]. These biofilm mats allow the effective colonization of the air–broth interface of static liquid culture, which does not occur in the absence of CLP. Some strains that defect from CLP production are able to invade and exploit cooperative mat populations owing to a growth rate advantage gained by defection. However, upon reaching high frequency, these cheaters impose a costly cheating load on the mat population by disrupting the mat structure and causing it to sink (P. Rainey, unpublished).

The natural challenge

The examples of social conflict reviewed here were all observed under laboratory conditions. In fact, among the published studies, only the *D. discoideum* strains had been recently isolated from the wild. Thus, the frequency and

nature of cheating in natural microbial habitats remains largely unexplored. Nonetheless, the readily detectable presence of defection in laboratory systems strongly suggests that social conflict in natural cooperative groups is commonplace.

Better characterization of cooperative microbial interactions is a prerequisite for deeper understanding of social conflict in either laboratory or natural environments. Any costly trait that helps to create a pool of publicly accessible resources can be considered cooperative. An incomplete list of common examples is given in Table 1. Some of these behaviours, such as myxobacterial predation in soil environments [40,49] are postulated to be synergistic types of cooperation (i.e. density-enhanced or group-limited cooperation), yet empirical support for such claims is often lacking. In many cases, the precise type of cooperation involved and what fitness benefits derive from it are not well defined. For example, it is often thought that auto-inducer secretion provides density-enhanced or group-limited benefits under natural conditions [7,50]. However, recently it has been suggested that many hypotheses about cooperative benefits of auto-inducer secretion have not been adequately tested and that such behaviour might simply reflect an individually advantageous ability to detect local diffusion rates under some natural conditions [8].

Basic questions about the cooperative nature of other microbial behaviours also require further investigation. For example, what is the effect of density (or lack thereof) on the growth rate of siderophore-producing bacteria under natural, iron-limited conditions? Do populations of any viruses grow faster within host cells at high MOI than in single-virus infections [51]? In rhizobia–legume symbioses, what are the precise evolutionary advantages of costly nitrogen fixation [52]? Finally, even the fitness benefits of social fruiting body construction by myxobacteria in the wild remain largely a matter of plausible speculation [53].

The emergence and maintenance of higher units of selection from groups of lower-level individuals (e.g. multicellular organisms from groups of unicellular organisms [17,54]) requires the mediation of conflict among those individuals [17,20,21,55]. In particular, if synergistic cooperation is to succeed, the negative group-level effects of cheating must somehow be constrained. Some mechanisms of limiting cheaters could be a function of population structure. The evolution of sophisticated cooperation might be less likely when relatedness within local groups is low. Thus, minimizing within-group conflict by establishing new groups from single individuals or small groups of clone mates (rather than by large, diverse groups in which cheaters are more likely to be present) might facilitate the evolution of cooperation. Understanding the fine-scale population genetic structure of microorganisms is crucial for defining the frameworks within which both cooperation and defection evolve.

Alternatively, cheating might be restrained by active policing [56], such as occurs among social insect workers [57]. In this case, policing individuals specifically inhibit cheater proliferation at the organismal level. Instances of microbial policing have not yet been reported.

Perspective

As expected from evolutionary theory, social conflict under laboratory conditions has been shown to occur in several distinct microbial cooperative systems. However, for many putatively cooperative microbes, understanding the precise nature and fitness benefits of their cooperation under natural conditions remains a major challenge. Such knowledge is important for measuring the degree to which defection is a 'tempting' evolutionary strategy in particular cases. Also unclear is the relative frequency of obligate versus facultative defection strategies among selfish microbes. Finally, mechanisms by which social defectors might be restrained in nature comprise an additional exciting area of future research for microbial behavioural ecologists.

Acknowledgements

I am grateful to Angus Buckling, Sebastian Bonhoeffer, Lee Kroos, Rosemary Redfield, Joan Strassmann, Michael Travisano, Paul Turner, David Sloan Wilson, laboratory members and an anonymous reviewer for helpful discussion and/or comments on this manuscript.

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