From quorum to cooperation: Lessons from bacterial sociality

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Abstract

The study of cooperation and altruism, almost since its inception, has been carried out without reference to the most numerous, diverse and very possibly most cooperative domain of life on the planet: bacteria. This is starting to change, for good reason. Far from being clonal loners, bacteria are highly social creatures capable of astonishingly complex collective behaviour that is mediated, as it is in colonial insects, by chemical communication. The article discusses recent experiments that explore different facets of current theories of the evolution and maintenance of cooperation using bacterial models. Not only do bacteria hold great promise as experimentally tractable, rapidly evolving systems for testing hypotheses, bacterial experiments have already raised interesting questions about the assumptions on which our current understanding of cooperation and altruism rests.

Keywords: Cooperation, altruism, kin selection, group selection, communication, cell-cell signalling

> One of the enduring unsettled issues of evolutionary biology is the paradox of collateral altruistic behaviour—that is, when some individuals subordinate their own interests and those of their immediate offspring in order to serve the interests of a larger group beyond offspring. (E.O. Wilson 2005, p. 159)
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> During the process of aggregation and early mound formation [in *Myxococcus xanthus*], 65 to 90% of the cells lyse [commit suicide]... (Dworkin 1996, p. 81)

Introduction

Given the intrinsic selfishness assumed to underlie Darwinian competition, cooperation should be rare in nature. But it is not. In fact, cooperation "pervades all levels of biological organization" (Sachs 2006, p. 1415), in the sense that individual entities act to produce an effect the cost of which to the agent is not immediately—or even ever—compensated. Not only do some organisms cooperate with other organisms (both of their own kind and of different kinds) and groups cooperate with groups, so also do spatially distant transcriptional units of DNA (‘genes’) cooperate to produce proteins; proteins cooperate to catalyze reactions and to transduce signals within cells; and cells cooperate with one another (Wingreen and Levin 2006). Without cooperation most of the “major landmarks in the diversification of life and the hierarchical organization of the living world” would have been impossible, including the transitions from nonlife to life, networks of cooperating genes to the first functioning cell, prokaryotes to eukaryotes, unicellular to multicellular organization, asexual to sexual reproduction, and so on to the development of complex ecosystems (Michod and Herron 2006, p. 1406). In short, the more nature yields her secrets, the more ubiquitous cooperation appears to be.

A growing number of reviews identifies multiple avenues to the evolution of cooperation and altruism in a selfish world (Dugatkin 2002; Kerr et al 2004; Sachs et al 2004; Fletcher and Zwick 2006; Lehmann and Keller 2006; van Baalen and Jansen 2006). How the paths are parsed varies among the different authors but, roughly speaking, are individuated according to the relation of cooperator to beneficiary and/or the allocation of costs and benefits associated with cooperative acts.

In *kin selection* (genic selectionism) cooperator and beneficiary are genetically closely related. Widely regarded as “one of the most important developments in evolutionary biology” (Griffin and West 2002, p. 15), W.D. Hamilton’s theory of inclusive fitness holds that nature favours a type of reproductive fitness that ‘includes’ both the fitness of an individual and the fitness of the individual’s close relatives (Hamilton 1964a, 1964b). Kin selection asserts that the genetic resources enabling cooperative or altruistic behaviour will evolve in groups where individuals are highly related and the cost of the cooperative action to the cooperator is relatively small while the benefit to kin is large (Maynard Smith 1964). In short, an organism is more likely to subordinate its own selfish interests to effective group behaviour if those with whom it cooperates share its genes and the personal fitness costs are not too high. Theoretically, the closer the relation (e.g., offspring, siblings), the more likely cooperative behaviour will evolve.

In *group selection* cooperator and beneficiary may or may not be genetically closely related but are part of a population that collectively exploits an ecological niche. *Reciprocity*, as the name suggests, means that the cost of a cooperative act is likely to be recompensed, directly or indirectly, by the beneficiary at a future time. Trivers (1971) proposed the concept of ‘reciprocal altruism’ in the context of the iterated Prisoner’s Dilemma of economic game theory to explain the evolution of cooperation among distantly related or unrelated organisms. Finally, mutual advantage resulting as an incidental benefit of the ordinary selfish behaviour of individuals is called *by-product mutualism*.[1]

Each of these paths has proponents and critics concerning its relative importance to the evolution of cooperation, generally and within particular contexts. For the past four decades, however, kin selection and the game theoretic interpretation of reciprocity have been the dominant theoretical paradigms (Sachs et al 2004). Both approaches assess acts in terms of costs and benefits, mainly to individuals, where cost and benefit are typically calculated in terms of reproductive fitness, its reduction or enhancement. Both approaches are also formally tractable—see, for example, Nowak's five mathematical "rules" for the evolution of cooperation (Nowak 2006)—and thus yield elegant computer simulations.

Kin selection and game theoretic reciprocity have always had their critics, but the limitations of these approaches have mounted with growing concern for ecological validity. A persistent complaint is that these abstract formal models often fail to connect with empirical observation because they do not account for complex and dynamic ecological factors, namely, "the 'real world' of existing biological organisms" (Leimar and Hammerstein 2006, p. 1403). Calculating costs and benefits to individuals is not always straightforward, especially in complex social arrangements such as class-structured[2] populations and multi-species consortia (Wild and Taylor 2006), neither is divining 'direct' and 'indirect' fitness components subject to selection (Wenseleers 2006). Recent kin selection modelling using non-linear cost/benefit functions believed better to mirror natural conditions yielded paradoxical results, for example, that there can be selection simultaneously for more *and* less cooperation (Doebeli and Hauert 2006).

Bacteria as experimental models of cooperation

If bridging the gap between theoretical and empirical research is, as Doebeli and Hauert (2005) suggest, "a major challenge for further progress in understanding the evolution of cooperation" (p. 761), then identifying model organisms of varying physiological and social complexity should be a high priority. Ideally, such models will be not only experimentally manageable but also well understood behaviourally, physiologically and genetically (Miklos 1993). Colonial insects, such as ants and bees, are well-studied systems of cooperative sociality. In recent years, however, interest has grown in microorganisms, including bacteria, as models of social evolution, cooperation and altruism (e.g., Strassmann et al. 2000; Velicer et al. 2000; Vulic and Kolter 2001; Griffin et al. 2004; Brockhurst et al. 2006).

On one hand, it could be argued such attention is overdue. Bacteria and archaea comprise the most numerous kingdoms of life and may account for the largest proportion of biomass on this planet, yet most theoretical biology and philosophy of biology has proceeded without reference to them (O'Malley and Dupré, in press). Certainly Darwin's theory of evolution by natural selection and the mid-twentieth century 'synthesis' that provided its modern form were advanced without reference to microbial data, which were then relatively limited. Over the past two decades rapid advances in microbiology due to increasingly sophisticated techniques for imaging the life-world of the very small have removed many barriers to observation *in situ* of unicellular individuals and populations. The exponential pace of bacterial reproduction under optimal growth conditions and the manageability of microbial genomes relative to those of multicellular organisms offer a potentially unique window into the processes underlying the evolution and maintenance of cooperative behaviours.

On the other hand, using bacteria[3] as models of cooperative behaviour may strike many as odd. Even scientifically sophisticated non-specialists tend to regard bacterial behaviour as fairly simple. As François Jacob put it: "One bacterium, one amoeba…what destiny could they have other than to form two bacteria, two amoebae…?" (cited in Dworkin 1985, p. 1). Bacteria are commonly viewed as clonal loners that reproduce at whatever pace the nutrient supply will allow until some environmental change overwhelms their limited, rigidly programmed adaptive moves. An artefact of the nineteenth century consensus in favour of a 'pure culture' methodology that (arguably necessarily) bracketed ecological realism (Dworkin 1985), this picture is now a caricature of the past (see especially Shapiro and Dworkin 1997; Watnick and Kolter 2000; Waters and Bassler 2005). Substantial evidence supports the view that communal living is the preferred bacterial lifestyle (Battin et al. 2007). Colonies can be comprised of one or more isolates, while surface-associated biofilms—such as plaque that forms on teeth—can be host to many hundreds (Kolenbrander et al. 2005). Most economically and medically significant microbial phenomena involve collective action, not merely large numbers. Growing appreciation of this fact has helped to shift attention away from bacteria grown as broth suspensions or colonies on agar to the dynamic, cooperative behaviours that predominate in nature.

As it does in human societies, cooperative behaviour in microbes serves a number of adaptive purposes (Shapiro 1998). First and foremost, collective behaviour provides *defence* against predators and unpredictable changes in environmental conditions, including the presence of antibiotics and host immune responses (Fux et al. 2005). Second, communal living optimises survival by providing access to resources and niches that individual cells cannot effectively obtain or utilize on their own, in many cases via *division of labour* (Kolter 2005). Third, group living may facilitate more efficient *genetic exchange* via lateral gene transfer (Molin and Tolker-Neilsen 2003), which can provide bacteria with innovative functional solutions to novel environmental problems (Ochman and Moran 2001). Cooperative behaviour in bacteria, as in animals, is facilitated by communication, which in microbes moderates genetic transcription (Miller and Bassler 2001). Sociality thus is a highly important—perhaps even pre-eminent—facet of microbial life, leading to suggestions for a 'sociomicrobiology' (Parsek and Greenberg 2005) or 'sociobacteriology' (see Shapiro, this volume).

The paper outlines three groups of cooperation experiments carried out with bacteria and discusses their implications for debates concerning factors that lead to and maintain cooperation and/or altruism in biological populations. These experiments—laboratory-based evolution of 'asocial' strains of *Myxococcus xanthus*, siderophore production in *Pseudomonas aeruginosa,* and character displacement in biofilms of *Pseudomonas fluorescens*—take place against the backdrop of a larger explanatory enterprise, evolutionary biology. Since Darwin's time one of the central issues of evolutionary theory has been the forces that shape genotypic and phenotypic diversification in adaptive radiation (Schluter and McPhail 1992). But it is far from easy to determine whether a particular trait evolved as an adaptive response to a particular selective context. The stately pace of evolution presents a major obstacle, the fossil record is notoriously full of gaps, and reconstructing ancestral environments is often a matter of creative interpretation of geological and climatic data. Thus it was possible for Gould and Lewontin (1979) to suggest that the scientific study of biological adaptation was "following a panglossian paradigm that promulgated 'just-so stories' rather than testing hypotheses" (Losos 2000, p. 5963). All three of the experiments described below shed light, some more directly than others, not only on microbial cooperative behaviour but also on the larger issues of adaptation.

According to Wingreen and Levin (2006), "Understanding cooperation among microorganisms presents conceptual and mathematical challenges at the interface of evolutionary biology and the theory of emergent properties of independent agents (p.1486)." I claim that it does more than that. Collectively, bacterial cooperation experiments illuminate gaps in dominant theories, provide support for minority theories, and pose new problems for research. For example, most theory and experiment of microbial cooperation has been guided by kin selection theory (Frank 1992, 1996; Brown 1999; Brown and Johnstone 2001; West and Buckling 2002; Strassmann et al. 2000), which some believe to be the sole basis on which cooperation can evolve among microbes (Keller and Surette 2006). As we will see in two of the three sets of experiments, this is not the case. Indeed, one recent study revealed a new mechanism for promoting cooperation and deflecting cheaters (Brockhurst et al. 2006), which may have macrobial analogues.

A terminological note before we proceed. While most have an intuitive notion of what cooperation means, ethologist Ronald Noë (2006) observes, in a review of cooperation experiments, that "chaos reigns in the terminology used" by the scientific disciplines concerned with cooperative behaviour (p. 1). These include ethology, behavioural ecology and evolutionary ecology as well as psychology, sociology, anthropology and economics—the latter four being traditionally concerned with human behaviour. Perhaps unavoidably, an investigator who takes the human case to be the benchmark of cooperation very likely will have a different view of what distinguishes the phenomenon from an investigator who looks first and foremost to biological and/or ecological considerations (Lyon 2006a). Consequently, some investigators insist an 'understanding' of the relationship between action and outcome is necessary for an agent to be described as truly cooperative, while Noë and others include organisms whose cooperative behaviour is ‘hard-wired’ and not just determined by “cognitive processes such as trial-and-error…learning, observational learning, insight and so forth” (Noë 2006, p.3).

Noë draws a definitive line between mere *sociality*, the tendency of conspecifics to aggregate and pursue their own interests in the context of a group (p.14), and *cooperation*, which requires an interaction or series of interactions between or among individuals that carries a cost for the agent but which, on average, results in a net gain for all participants of the interaction (p.4). Noë intends this reading of cooperation to include “all other terms that have been used for mutually rewarding interactions and relationships: reciprocity, reciprocal altruism, mutualism, symbiosis, collective action and so forth” (p.4), and it will be adopted here. *Altruism*, by contrast, characterizes action by one individual that benefits another (or group) at the cost of the agent’s own survival, wellbeing or reproduction.

Finally, a word about what will not be addressed here. ‘Directed reciprocation’, defined as “cooperation with individuals that return benefits” (Sachs et al. 2004, p. 137), underlies symbiotic mutualisms where partners are specifically chosen or are coupled such that the fitness of one affects the fitness of the other. Bacterial mutualisms of this sort abound in nature. Perhaps the most extensive functional dependency identified to date was recently discovered in a sediment-dwelling marine worm, *Olavius algarvensis*, a distant relative of the earthworm that lacks a mouth, digestive tract or a typical excretory apparatus (Stahl and Davidson 2006). Five different bacterial symbionts provide the worm with multiple sources of nutrients in an accessible form and take up and recycle the worm’s waste products. Such mutualisms have not been the focus of cooperation experiments to date, however, and will not be discussed. Neither will be issue of kin recognition. Genic selection has long been assumed to drive cooperation and altruism in insects and simple vertebrates on the basis it is the least cognitively demanding. The capacity to discriminate relatives from non-relatives is a potent factor for promoting cooperation by kin selection but is not imperative. Limited dispersal of offspring also ensures that neighbours are likely to be close relatives. Reciprocal altruism, on the other hand, presents unavoidable cognitive demands, which is why it is considered an important evolutionary ratchet of human cognition (Sterelny 2003). To become evolutionarily stable, the ‘tit-for-tat’ strategy and its variations seem minimally to require recognition of who does or does not reciprocate cooperative behaviours. Whether bacteria and unicellular eukaryotes are capable of recognizing kin remains an open question. I have argued elsewhere that recent developments in microbiology undermine standard arguments against bacterial cognition and a closer look at bacterial behaviour would reward cognitive scientists (Lyon 2006b; see also Shapiro, this volume). As no cooperation experiments published to date have probed kin recognition in microbes, these arguments will not be considered.

1. Mutation experiments in *M. xanthus*

Myxobacteria are especially well suited to cooperation experiments and were the first used to study the evolutionary genetics of microbial social behaviour (Velicer et al. 2000; Velicer et al. 2002). A Gram-negative group of proteobacteria that lives in habitats rich in organic nutrients, myxobacteria have a complex life-cycle that appears to be social at every stage (Shimkets and Dworkin 1997). *M. xanthus* has one of the largest known bacterial genomes, a substantial proportion of which underlies social behaviour (Velicer et al. 2006). Much of this behaviour relates to the fact that *M. xanthus* is a wily predator and displays many characteristics of predatory animals: aggressive territoriality (Shimkets and Dworkin 1997), deceptive entrapment of prey species (Shi and Zusman 1993), and 'pack' hunting compared to that of wolves (Dworkin 1973). Under growth conditions, *M. xanthus* move as coordinated swarms, which secrete extracellular antibiotics to break open (lyse) microbial prey and other enzymes to degrade and digest them (Spormann 1999, p. 622).

Under starvation conditions, *M. xanthus* perform one of the most dramatic bacterial behaviours known: a multi-stage developmental cycle leading to sporulation involving aggregation of hundreds of thousands of cells and formation of a fruiting body comprised of differentiated cell types. In a process likened to "the great animal herd migrations" up to a million cells move toward aggregation sites where fruiting bodies form (Shimkets 1999, p. 526). Of the initial cell population, only 10-20% will transform into long-lasting, stress-resistant myxospores and survive to reproduce another day. A staggering 65-90% of the initial population collectively suicide, by rupturing their cell envelope (autolysis) (Dworkin 1996). The function of this sacrifice has not been unequivocally established, but the cell fragments are assumed to provide carbon and energy for development. Another 10% of the population transform into special cells that remain on the periphery (Shimkets 1999). Their function is unclear, but given the territoriality of myxobacteria it has been suggested that these cells could be a kind of sentry, to prevent pillaging of the sacrificial feast and predation of the dormant myxospores. After all, starvation is the stimulus for the sequence.

In the late 1990s geneticist Gregory Velicer and colleagues began a program of laboratory-based evolution using clones of a standard strain of *M. xanthus* (Velicer et al. 2000). The idea was to see what would happen if normally social bacteria were subjected to a selective regime favouring competition over many generations. The findings might shed light on several questions in evolutionary biology, not only the nature and genetics of myxobacterial cooperative behaviour. Twelve experimental lineages were developed from the ancestral strain, which then were grown in a rich nutrient medium for 1000 generations. Cooperative myxobacterial behaviour appears to be an adaptation to fluctuating nutrient availability, so the researchers predicted that socially deficient strains—'cheaters' in game theoretic parlance—would evolve in optimal growth conditions. Significantly, all 12 lineages developed partial or complete deficits in social competencies, related to motility and/or development.

One lab-evolved isolate, dubbed “Obligate Cheater” (OC), was unable to form myxospores except in the company of socially proficient cells. When mixed with cooperators, OC sporulated more efficiently and was over-represented in the fruiting body (Velicer et al. 2000). Another isolate that had completely lost its ability to engage in social motility, necessary for fruiting body formation, *regained* this capacity when mixed with proficient cells (Velicer et al. 2002), a process called extracellular complementation, about which more in a moment. The authors consider the motility experiments particularly important because they show that the loss of S-motility is an adaptation to asocial conditions. When returned to the asocial conditions of their evolution, ‘rescued’ S-motile cells were not as competitive.

Perhaps the most interesting result to date has been the *re-evolution* of sociality in OC cells in a form superior to its ancestral lineage. To see how OC fared when it constitutes the minority in a cooperative population, the researchers innoculated a population of a marked isolate of the ancestral strain with 1% OC cells, known as an ‘invasion-from-rare’ experiment. The chimeric population was grown through six alternating cycles of starvation and growth. After four cycles OC had re-evolved the ability to undergo social development (Fiegna and Velicer 2005). By the end of the experiment the resulting OC-turned-cooperator—a genetically distinct isolate called PX, or Phoenix—out-competed its own cheating ancestor, OC, and was much less susceptible to invasion than the ancestral lineage (Fiegna et al. 2006). Moreover, the transition from cheater to super-social appears to involve a single mutation to a gene that expresses an acetyltransferase, the precise biological function of which is unknown in this organism (Velicer et al. 2006). Transferases, as a molecular class, are proteins that attach chemical moeities (in this case an acetyl group) onto another protein, which activates or inhibits a signalling cascade.

In short, as expected under traditional evolutionary theory, the experiments show that a social bacterium, subjected over many generations to growth conditions favouring individual competition, can lose the ability to participate in cooperative behaviours as a result of adaptation to asocial conditions, yet develop a superior capacity to sporulate in the company of cooperators. Exposed to the conditions under which ancestral cooperation is assumed to have evolved, however, asocial cheaters do not come to dominate cooperative populations as autonomous competitors, as might be expected, but re-evolve the ability to cooperate. Moreover, neo-cooperative cells are competitively superior, which suggests that super-social cells may eventually come to dominate a genetically mixed population. Neither of these outcomes—re-evolution of social competence and super-sociality—appears to fit standard interpretations of natural selection and kin selection. The experiments do show, however, that obligate dependence on an altruistic host is not a terminal condition, but can be "an evolutionary stepping stone" to new modes of existence (Fiegna et al. 2006, p. 310).

The myxobacterial experiments provide support for some predictions of kin selection theory, however. The interactions of socially proficient cells from nine naturally occurring isolates of *M. xanthus*, taken from spatially distant locations, were compared to see how well—or even if—they would cooperate with other genotypes (Fiegna and Velicer 2005). Noncooperation can be exhibited as exploitation or "outright antagonism" (p. 1980). Cells from the nine strains were mixed in all possible pair-wise combinations and subjected to starvation conditions. The experiments provide the first evidence of facultative cheating (exploitation) in *natural* social isolates. They also show that selfish competition in a social microbe can be an evolutionary dead-end. All of the mixed strains produced fewer fruiting bodies and had lower overall productivity than clonal populations; in one antagonistic pair spore production almost collapsed, falling by 90% (Gross 2005). As kin selection theory might predict, *M. xanthus* appears to have "diverged into a large number of distinct social types that cooperate with clone-mates but exhibit intense antagonism toward distinct social types of the same species" (Fiegna and Velicer 2005, p. 1980),

The lessons of the *M. xanthus* experiments for understanding of cooperation are at least three. First, bacteria are excellent models for demonstrating adaptation under particular selective regimes. Subjected to environmental conditions that do not require cooperation, social deficits become common; all of the experimental lineages developed such deficits. Second, cheaters may be reproductively superior but this does not ensure that they will come to dominate a cooperative group, which suggests other factors must be contributing to the maintenance of cooperation. Finally, within highly cooperative species natural selection appears to favour the strengthening of sociality rather than individual autonomy, demonstrated by the re-evolution of social proficiency by an obligate cheater.

As impressive as these experiments are, I have misgivings about applying genic selectionist models, and especially a game theoretic vocabulary, to bacterial behaviour in general and *M. xanthus* in particular. The first qualm relates to extracellular complementation, the well-established phenomenon of myxobacteria actively compensating for the genetic shortcomings of their fellows (Shimkets 1999, p. 528). This is the process that 'rescued' S-motility in Velicer's deficient strains. It has been widely supposed that complementation results from the secretion of molecules by proficient cells that are taken up by deficient cells. Recent studies of *M. xanthus* mutants deficient in proteins necessary for gliding, a form of social motility, suggest that the process may be more active in some cases. One form of complementation of the S-motility mutants occurs when "donor and recipient briefly fuse their outer membranes" for direct transfer of the competence factor (Nudleman et al. 2005, p. 125). Competent individuals thus actively transfer part of themselves to their deficient fellows, enabling their participation in a cooperative behaviour. The question then arises of how notions of ‘cooperators’ and ‘cheaters’ apply in a situation in which one organism actively compensates for the deficiencies of another? Complementation could be viewed as a form of ‘policing’ to prevent free-riding, but it appears very costly to the disciplinarian. Whether kin selection is adequate to explain complementation remains to be seen.

What at first glance may look like a cheating strategy may not be. During the extended sporulation sequence in *Bacillus subtilis*, for example, some individuals not only delay their own transformation but also release antibiotics to kill their sisters, whose lysed remains they cannibalise (Gonzalez-Pastor et al. 2003). On the face of it, initiation of sporulation appears to be a stimulus for developmental cheating. Appearances may be deceiving, however. Sporulation in *B. subtilis* is an elaborate process that takes up to 10 hours and involves the transformation of a growing cell into a type that can remain dormant for many years. Spore formation is thus a “life-or-death” decision (Bassler and Losick 2006, p. 242). The cannibalism phase of sporulation appears to be a bistable switch that keeps the colony’s options open as long as possible. Only when “no siblings remain to be cannibalised and no other sources of nutrients become available” does the colony’s development progress to the point of no return (p. 243). In short, developmental cannibalism does not equate with developmental cheating; rather, it is an altruistic act that benefits the group.

A second misgiving concerns the prevalence of lateral gene transfer both within and between bacterial species (but not, apparently, within *M. xanthus*) and even across kingdoms, bacteria-to-plant transfers being the best documented (Mathesius et al. 2003). Lateral gene transfer is the acquisition of foreign DNA, either by taking it up from the environment (transformation), receiving it from another bacterium in a complex operation in which DNA passes from one cell to another (conjugation, or sex), or by viral transmission. Lateral gene transfer can confer novel functional competencies, such as antibiotic resistance, and is now assumed to be a major mechanism of bacterial evolution (Ochman and Moran 2001). Presumably, the ability to promiscuously donate or receive genetic material across genera, families and kingdoms skews the notion of ‘kin’ somewhat. Are kin those that share adventitiously acquired plasmids (small cassettes of DNA) or most of the genome? If selection is on the basis of the chromosome rather than plasmids, what proportion of the genome must be shared to count as ‘close’ kin? Are housekeeping genes enough? Is there a diagnostic marker—a so-called ‘greenbeard’ trait? If so, how is it detected? This raises the question of microbial kin recognition. Answers to these questions, “unconsidered by current theory” (Brockhurst et al 2006, p. 2033), will require detailed investigation.

2. Siderophore production in *Pseudomonas aeruginosa*

Many medically and economically important conditions involve multiple types of bacteria, a situation that raises questions about the effect of multi-parasite infections on virulence. Steven Frank (1992, 1996, 1998) developed an influential model, based on kin selection theory, that predicts infection by several species will reduce the population's overall genetic relatedness, thus weaking the main factor supporting cooperation, and thus lead to more intense competition for resources. Greater resource competition means greater virulence. Therefore, infection by multiple isolates will be more virulent. Although the evolutionary logic was widely accepted, few empirical data supported these predictions. Indeed, several studies suggested the opposite: multi-parasite infections tended to be less, rather than more, virulent.

West and Buckling (2003) advanced an alternative model that predicted virulence would be stronger, not weaker, where relatedness was high, provided the strain was cooperative. They cited as an example mechanisms for scavening iron from a host. Siderophores are small iron-binding molecules secreted by bacteria in response to iron deficiency. Iron is necessary for microbial growth but is hard to access within animal hosts, whose immune defences often actively withhold the mineral. Iron-bound siderophores can be taken up by all group members, not just those who produce them, so siderophore production is a "'whole group' cooperative trait," costly for the individual but beneficial for the group (Griffin et al. 2004, p. 1025). If an infecting strain cooperatively produces siderophores—and, if they cooperated, they would be highly related according to kin selection theory—then access to iron would confer a growth advantage enabling better host exploitation and, thereby, increasing virulence (West and Buckling 2003).

There is a problem with this interpretation, however. Higher relatedness within a group may promote greater cooperation, but if resources are limited and so too is dispersal, then relatives become competitors, and competition will increase as resources are depleted. What happens then? Hamilton believed that, where dispersal is limited, the accumulation of genetic similarity within a population would "favour a less targeted kind of altruism towards neighbours in general" and expand the range of cooperation beyond close relatives (Queller 2004, p. 975). By contrast, Frank (1998) predicted that as local competition intensified the influence of relatedness on selection for cooperation would decline and cooperation would be selected *against*. Frank's predictions were tough to test experimentally not merely because of the pace of evolution but the difficulty of teasing apart the variables, relatedness and the scale of competition (Queller 2004). A bacterial model not only provided a way to observe evolution over many generations, but also, perhaps more importantly, allowed researchers to independently manipulate the variables of relatedness and competitive scale by using different clonal populations in differing ratios and different competitive settings.

Griffin, West and Buckling (2005) tested Hamilton's and Frank's hypotheses using siderophore production in *P. aeruginosa* as the target cooperative behaviour. A Gram-negative proteobacterium, *P. aeruginosa* is a highly versatile opportunist capable of infecting an impressive variety of plant and animal hosts—it is even capable of growing in diesel and jet fuel—and thus provides an example of another evolutionary paradox posed by bacteria. Pathogens are assumed to be under selection pressure to co-evolve with their hosts, so natural selection should favour single-host specialization. Yet specialist pathogens are relatively rare, and multi-host generalists like *P. aeruginosa* are common (Woolhouse et al. 2001). A high level of genetic diversity, from high mutation rates or rampant gene transfer, is presumed to predispose pathogens to generalism. The ability to cooperate may be another. Gene transfer and the production of siderophore, virulence factors and biofilms are among a number of cooperative traits in *P. aeruginosa*, which appears to be every bit as cooperative as *M. xanthus*, even if its behaviour is not so overtly dramatic.

The 'cooperators' in the siderophore experiment were a wild-type strain that produces the iron-binding agent pyoverdin; the 'cheaters' were from a mutant laboratory strain that cannot. Relatedness was measured on the basis of the altruistic allele for pyoverdin production. A two-factor analysis of variance design resulted in four treatments, each of which was run four times, totalling 16 experiments. The results were, quite literally, visible. Pyoverdin is green, so cooperator-dominant colonies took on that colour, while cheater-dominant colonies are white.

Consistent with Hamilton's rule, the study showed that cooperation evolves to a greater extent in groups where relatedness is high. However, as Frank predicts, the importance of relatedness declines as local competition intensifies. The experiments show that close genetic relatedness can crucially influence the evolution of cooperation but its effects are relativistic, modulated by the scale of competition. "Local competition [in the siderophore experiments] exactly cancelled out the effect of high relatedness" (Queller 2004, p. 976).

Unexpectedly, the study also suggests that cooperators are relatively *advantaged* by global competition, even when relatedness is low. While not explicitly designed to test group selection theory, the results of the siderophore experiments nevertheless provide powerful support for its central thesis, summed up by longtime advocate David Sloan Wilson as "selfishness beats altruism within a group, [but] altruistic groups trump selfish groups" (Brown 2003). In his commentary for *Nature*, David Queller describes the siderophore experiments as "the most basic group-selection experiment possible":

> The conditions of low and high relatedness correspond exactly to the presence and absence of within-group selection. The conditions of global and local competition correspond exactly to the presence and absence of between-group selection…The

> results confirm that cooperation is favoured by between-group selection and disfavoured by within-group selection. (Queller 2004, p. 976)

The result is significant because group selection is still controversial. Contribution to “the good of the species” was the chief explanation for cooperative and altruistic behaviour before the introduction of Hamilton’s rule (Sachs et al. 2004, p. 136), but in its wake group selection became a heretical theory, despite the efforts of Wilson (1977) in particular. Group selection nevertheless is gaining adherents, particularly among pluralists advocating multiple levels and/or interacting mechanisms of selection (e.g., Sober and Wilson 1998). Moreover, group selection is the central pillar of a new model of eusociality (Wilson and Hölldobler 2005) to which the results of the siderophore study nicely conform, although bacteria are not typically regarded as eusocial. Consistent with the siderophore findings, the Wilson-Hölldobler model holds that kinship has a relativistic effect the evolution of eusociality; it can promote or undermine, depending on other factors.

3. Biofilm formation and character displacement in *Pseudomonas fluorescens*

A modified version of the question at the heart of the siderophore study—what happens to cooperation when competition among kin increases—has long occupied evolutionary theorists. What happens when the ecological niches of two closely related species overlap, and they compete for resources? In a seminal paper Brown and Wilson (1956) introduced the concept of *character displacement* to describe “a seldom-recognized and poorly known speciation phenomenon […] of potential major significance” (p.49). The basic idea is that, in the zone where niches overlap and closely-related species compete for resources, the differences between them will be accentuated and their phenotypes will diverge, thus lessening competition. Competition declines because character displacement enables the exploitation of new resources. The ‘displaced’ characters may be morphological, ecological, physiological or behavioural, but natural selection will favour divergence if it has a genetic basis, Brown and Wilson argued. Character displacement thus lies at the heart of the debate over the role of competition in structuring ecological communities (Dayan and Simberloff 2005). The problem, as always, is that “interspecific competition of the direct, conspicuous, unequivocal kind” is just as “difficult to catch and record” as the development of reproductive barriers between newly diverging species (Brown and Wilson 1956, p.60).

Enter *P. fluorescens*, a relative of *P. aeruginosa.* In a spatially heterogeneous environment, the ancestral smooth (SM) genotype of *P. fluorescens* is known to diversify through mutation into “a range of niche-specialist genotypes that are maintained by negative frequency-dependent selection” (Brockhurst et al 2006, p. 2030). Frequency-dependent selection is the evolutionary process whereby the fitness of a phenotype depends on its frequency relative to other phenotypes in a population. In negative frequency-dependent selection, the fitness of a phenotype increases as it becomes less common. For example, a rare strain of flu virus is more fit (i.e., is better able to reproduce) because the host population has not developed immunity to it. Among the niche specialists that appear in experiments with *P. fluorescens*, one mutation routinely becomes ecologically dominant—perhaps surprisingly, on current theories of cooperation. Wrinkly spreader (WS), a mutation that over-produces a cellulose-based polymer, is capable of forming a biofilm at the surface of the nutrient broth. Over-production of the material is costly to the individual, but the biofilm provides a group benefit because colonization of the air-broth niche allows better access to oxygen. SM genotypes benefit from inhabiting the biofilm while making no contribution to its integrity, which in this context makes them cheats. Indeed, if SM numbers increase disproportionately, “the biofilm sinks under their uncooperative weight” (Brown 2006, p. R960). On the other hand, several different types of WS have been known to evolve and co-exist in WS colonies. This raises the following questions: Are these phenotypic divergences adaptations, and how do they affect cooperative biofilm formation?

Brockhurst and colleagues allowed nine populations of equal numbers of a standard strain and a marked strain of *P. fluorescens* to evolve for six days, at the end of which a single WS colony was grown from cells of each marker type isolated at random from each of the nine populations. If diversification for using different resources had occurred as a result of competition in the biofilm, the researchers reasoned, then *co-evolved* pairs of WS should display reciprocal negative frequency dependence (RNFD), that is, their fitness would increase as they both became less common. *Random* pairs of WS competing for the same resources would not display RNFD to the same extent. To test this hypothesis, invasion-from-rare experiments were run for co-evolved and random pairs of WS. The results supported the hypothesis that character displacement had occurred under competitive pressure. All nine of the co-evolved pairs displayed RNFD and partitioned the biofilm, although the magnitude of diversity varied between groups. Only five of the nine random pairs displayed RNFD, and at a significantly lower rate compared to the co-evolved pairs.

The researchers next asked whether phenotypic divergence within the biofilm affects its susceptibility to invasion by cheats. Microcosms of the original strains were innoculated in two different treatments, with WS cells grown in monocultures and with mixtures of equal parts of the co-evolved WS pairs. After six days of incubation, the proportion of SM colonies was measured. As predicted, the proportion of SM cheats in populations innoculated with the mixed co-evolved WS pairs was significantly lower than in the populations innoculated with the monocultures, “indicating that the observed adaptive character displacement prevented the establishment of the cheating SM phenotype” (Brockhurst et al. 2006, p. 2031). Additional experiments showed that not only were highly diversified populations less susceptible to invasion, they were also more productive.

This series of experiments is important for several reasons. First, with regard to the broader concerns of evolutionary theory, the experiments constitute a manipulable evolutionary context that provides empirical evidence for character displacement—an idea that since its introduction has gone from high fashion to marginality to renewed respectability (Dayan and Simberloff 2005). Moreover, the bacterial experiments appear to meet all extant criteria for testing hypotheses of ecological character displacement (Losos 2000), a rare feat for studies of macroscopic organisms (e.g., birds, frogs, ants, beetles) for which the criteria were devised. Second, relative to the evolution of cooperation, the experiments provide evidence for a "novel mechanism for maintaining cooperation in the face of local competition" and in the absence of mechanisms, such as policing, for the active repression of competition. This suggests that "cooperation might be more resistant to the effects of individual selection…[and] conditions favouring the maintenance of cooperation are much broader" than previously thought (Brockhurst et al. 2006, p. 2032). While the results seems to suggest that cooperation is favoured and maintained not only by competition between groups but also within groups, the researchers say the experiments do not challenge group selection, but, rather, demonstrate that under "certain conditions, where local competition results in diversification, the benefits of diversity may exceed the costs of cheating and favour the spread of cooperation" (Brockhurst et al. 2006, p. 2032).

Third, regarding virulence prediction, the experiments suggest that extrapolating "a general rule" for virulence in multi-parasite infections may be futile (Brockhurst et al. 2006, p. 2032). This is because the reduction of relatedness among cooperating individuals for one phenotypic trait (exploitation of the ecological niche) but not another (the cooperative trait) may increase the overall fitness of a cooperating group, thereby increasing virulence.

Finally, the experiments are a likely first step toward teasing out the complex issue of partitioning in biofilms, a socially important phenomenon that is extremely costly in terms of human health and economic life. Biofilms are large, three-dimensional aggregates of bacteria—usually comprised of multiple metabolically diverse species but sometimes hundreds of them—which adhere to surfaces in moist or watery environments, such as soils, teeth, living tissue, medical implants, air conditioning systems, pipelines, sewage treatment plants, and marine equipment; in short, just about any sort of surface in an aqueous environment (Costerton et al. 1987). Watnick and Kolter (2000) have compared a biofilm to a city, and the analogy does not appear to be over-stretched. Video evidence shows that biofilms are loci of both growth and recruitment, highly dynamic structures constructed and sustained by the 'commitment' of individual bacteria, which often undergo dramatic changes in cell morphology or function (Kolter 2005). Individual cells entering the biofilm seem to "distribute themselves according to who can survive best in the particular microenvironment and also based on symbiotic relationships between groups of bacteria" (p. 2676). Here is microbiologist William Costerton, whose research team coined the term ‘biofilm’ in 1978, giving a bacterium’s eye view of such a structure:

> If you found yourself in a biofilm, you’d be going along a channel full of water, like the canals in Venice, and up from the bottom of the channel, on either side, would be these slime towers. The channels would be bringing in oxygen and nutrients, and removing waste. And within each building, so to speak, some of the bacteria would be cooperating with each other, making one compound and passing it along to the next. (Chicurel 2000, p. 284)

Although it remains to be seen whether any bacterial isolates can properly be characterized as eusocial, the most extreme type of cooperation, the parallels between the cooperative behaviour of some types of bacteria and eusocial insects are striking. Wilson and Hölldobler (2005) identify three “key adaptations” for the evolution of eusociality: aggregation for defence, division of labour and communication. All three are found in bacteria, sometimes within a single isolate. As we have seen *M. xanthus* provides examples of aggregation for defence and division of labour. Biofilm formation is also believed to be primarily defensive and, in multispecies consortia, to involve divisions of labour (Costerton et al. 1987). Biofilms are resistant to many toxic substances, such as antibiotics, chlorine, and detergents (Costerton et al. 1987); to predation by protozoa and bacteriophage (Matz et al. 2004); and to attack by host immune defences (Fux et al. 2005). A recent study found that biofilm-based cells of *Campylobacter jejuni* live twice as long at ambient temperature and atmosphere as free-living cells (Joshua et al. 2006). Moroever, just as ants and other colonial insects rely on chemical communication, so do fruiting body formation in *M. xanthus* and biofilm formation in Gram-negative bacteria such as *P. aeruginosa*.

4. Cell-cell communication and cooperative behaviour

Although cell-cell signalling was not a variable in the cooperation experiments described above, systems of chemical communication are known to be involved in all three of the cooperative behaviours investigated: fruiting body formation in *M. xanthus* (Kaiser 2004), siderophore production in *P. aeruginosa* (Stinzi et al 1998; Ren et al. 2005); and biofilm formation in *P. fluorescens* (O’Toole et al 2000; Wei and Zhang 2006). So similar is the trajectory of *M. xanthus* fruiting body formation to that of biofilm construction in *Pseudomonas* (both *aeruginosa* and *fluorescens*)—including the crucial role of cell-cell signalling—O’Toole and colleagues (2000) suggest they both may serve as models of the same basic developmental process, although they are triggered under different environmental condition.[4]

Just as microbiologists found it difficult to accept cooperative sociality in bacteria (Shapiro and Dworkin 1997), they initially regarded cell-cell communication as an anomaly specific to bioluminescent bacteria (Miller and Bassler 2001). Today the consensus is that cell-

cell communication is widespread in the bacterial kingdom (Kolter 2005), although sceptics remain (Redfield 2002)[5] and others suggest that claims about the ubiquity and importance of bacterial communication are exaggerated (Keller and Surette 2005).[6] As it is in social invertebrates and vertebrates, communication appears to be involved in a wide variety of complex social and cooperative behaviours in bacteria (Bassler and Losick 2006), including: 1) some forms of social *motility*, such as swarming in *M. xanthus* (Daniels et al. 2004); 2) production of *secondary metabolites*, such as virulence factors, bacteriocins,[7] toxins, bioluminescence, degradative enzymes, exopolysaccharides, and pigments (von Bodman et al. 2003); 3) *global changes of cell state*, such as the transition (in some species) from exponential growth to the stationary phase (Lazazzera 2000), and (in others) the initiation of chromosomal replication (Withers and Nordstrom 1998); 4) *lateral gene transfer* (Lanka and Pansegrau 1999); 5) *symbiotic mutualism* (Visick and McFall-Ngai 2000); 6) *pathogenic infection* (Donabedian 2003); 7) *multicellular developmental stages*, such as fruiting body formation in *M. xanthus* (Kaiser 2004); and 8) *biofilm* formation, maturation and dispersal (Kjelleberg and Molin 2002).

Cell-cell signalling is the ability of one cell, or a group of cells, to regulate the physiology (via gene expression) and influence the behaviour of other cells by any actively or passively transported bacterial product.[8] The technical term for bacterial communication is *autoinduction*, so-named because the organism produces a class of molecule (autoinducers) that stimulates a change in genetic expression in itself as well as organisms of the same kind (Miller and Bassler 2001). The change may result in the production of another class of molecule that performs some function—virulence factors, for example—or a complex regulatory cascade that leads to a global transformation of the cell, as in sporulation. The label *quorum sensing*—as in, *Is there a quorum for effective action?*—was adopted when it became apparent that genetic changes were induced at threshold concentrations, which depends on population density. The supposition is that quorum sensing ensures that individuals do not engage in behaviours that are too costly and unproductive when undertaken by one or just a few cells (Bassler 2002). A few cells releasing a virulence factor will swiftly be attacked by a host immune system; bioluminescent molecules secreted by a lone symbiont will not be visible.

Autoinduction is viewed predominantly as a mechanism for determining population density because activation (or inhibition) of QS-related genetic transcription is threshold-dependent. However, researchers increasingly suspect that census-taking is not all that QS systems do. Whole-genome studies suggest that quorum sensing not only provides bacteria with a mechanism for initiating cooperative behaviour but also for alternating “between distinct genome-wide programs” customized for a particular lifestyle, social or solitary (Waters and Bassler 2005:332). The discovery that QS signalling can repress global regulatory circuits as well as activate them suggests that terminating the processes needed for autonomous living is as important as coordinating group behaviour.

At least five types of QS system have been identified to date: three ‘archetypal’ systems (two different systems common to Gram-positive and Gram-negative bacteria respectively, and a third found in both types) (Miller and Bassler 2001); a fourth system (so far) unique to *M. xanthus*; and a fifth “conversational” system recently discovered in *P. aeruginosa* (Deziel et al. 2004). At time of writing at least three other types of autoinducing molecules had been identified (Waters and Bassler 2005), and there is no reason to presume this will be the end of discovery. Most QS molecules are species-specific, enabling intraspecies communication. However, one system (AI-2), found in a substantial portion of the Gram-negative and Gram-positive isolates sequenced to date, may be a sort of “bacterial Esperanto”, facilitating communication between species (Bassler 1999, p. 584). AI-2 has been implicated in bioluminescence, virulence, siderophore production, bacteriocin production, motility, and mixed-species biofilm formation.

While the molecular details differ, the key functional components of quorum sensing appear to be the same across the bacterial domain: signal generation, signal perception, signal transduction and genetic transcription (Zhang 2003). The ‘overall mechanism’[9] among the various systems also appears to be roughly similar (Pasmore and Costeron 2003): the autoinducer is synthesized and exported from the cell by diffusion or secretion. The molecules diffuse away from the cell in most environments and, owing to a moderate half-life, don’t usually build up. When cell densities are high, however, the molecules reach a threshold value that activates genetic transcription for a change in behaviour—for example, the production of a secondary metabolite, such as an iron-scavenging siderophore, or exopolysaccharide, the ‘glue’ that holds cells in a biofilm together.

Many species operate two autoinduction systems, but an increasing number of bacteria, like *P. aeruginosa*, have been found to use three systems, and that may not be the limit. Three types of “network architectures” have been identified for the operation of multiple QS systems: parallel, serial and antagonistic (Waters and Bassler 2005). Parallel systems, identified in *Vibrio*, may serve to filter out noise, either from non-signalling molecules in the environment or from signal mimics, operating much like multiple signatories on a bank account (Taga and Bassler 2003). By contrast, the QS systems in *P. aeruginosa* act sequentially, enabling the expression of different virulence factors at different times and stages of infection. In *B. subtilis* the QS network is based on two peptides that operate antagonistically, stimulating depending on context one of two mutually exclusive developmental programs: competence (the capacity to take up DNA from the environment) or sporulation (Solomon, Lazazzera, and Grossman 1996).

As a key mediator of bacterial cooperative behaviour, quorum sensing is a target for friends and foes, both prokaryotic and eukaryotic (Bauer and Robinson 2002). Bacterial interference with quorum sensing—called *quorum quenching* (Dong et al. 2001)—includes altering or removing signals and/or inhibiting their synthesis. Quorum quenching may help secure competitive advantage within an ecological niche or provide self-defence against another isolate's virulence factors or bacteriocins, which are released at density. Many plants have evolved strategies for disrupting or manipulating quorum sensing. A QS 'mimic' was first discovered in the Australian seaweed *Delisea pulchra*, which secretes furanones to prevent bacterial colonization (Givskov et al. 1996). Whereas *D. pulchra* produces dozens of QS mimics, all inhibitory, other organisms (e.g., *Chlamydomonas reinhardtii*) produce mimics that stimulate bacterial QS-related activities (Teplitski et al. 2004), while still others produce both stimulatory and inhibitory mimics (Gao et al. 2003). One type of bacterial autoinducer globally influences the expression patterns of over 150 proteins in the legume *Medicago truncatula* (Mathesius et al. 2003).

In short, cell-cell communication appears to be extremely important to bacterial cooperative behaviour and, given the richness of molecular diversity, may have evolved independently several times. Because quorum sensing allows groups of bacteria, including groups of mixed bacteria, to behave effectively as a multicellular organism, a growing number of researchers consider intercellular signalling to be central to understanding the evolutionary transition from unicellular to multicellular life (Costerton et al. 1987; Dworkin 1996; Shapiro 1998; Miller and Bassler 2001). Phylogenetic comparisons suggest that quorum sensing is not a recent innovation but "originated very early in the evolution of the Gram-negative proteobacteria" (von Bodman et al. 2003, p. 471). The finding that genetic sequences underlying quorum sensing are highly conserved dovetails with growing evidence that cooperation, too, may be an ancient survival strategy. Consensus is forming that stromatolites, the rocky marine formations on the West Australian coast, were formed in part by the action of ancient microbes, possibly gliding filamentous cyanobacteria, which aggregated into something like the biofilms of today (Allwood et al. 2006). These structures are 3.4 billion years old. This is not to suggest that ancient microbes, such as those believed to have formed stromatolites, employed quorum sensing as their contemporary descendants do. Nevertheless, the antiquity of the mechanism believed essential for stimulating and coordinating much cooperative behaviour among contemporary bacteria offers support for the view that stromatolites could have been produced by microbial social behaviour.

Conclusion

The lessons of bacterial experiments for understanding cooperation are several. First, the *M. xanthus* experiments. Importantly for evolutionary theory generally, these experiments provide empirical evidence of adaptation under a particular selective regime, in this case, social bacteria evolving in an environment that does not require cooperative behaviour to maintain or enhance individual fitness. Next, important to the study of cooperation, the *M. xanthus* experiments demonstrate that cheaters may be reproductively superior under certain conditions, but this does not ensure that they will come to dominate a cooperative group. Indeed, within this highly cooperative species natural selection appears to favour the strengthening of sociality rather than individual autonomy. Thus factors besides kinship must be contributing to the maintenance of cooperation. The siderophore experiments confirm that kinship supports cooperation but also that the effects of genetic relatedness are attenuated as local competition among kin increases. Kin selection thus appears to be a relativistic factor in highly cooperative groups. On the other hand, group selection, a still-controversial theory, received strong support. Both findings conform to the Wilson-Hölldobler model of eusociality. Finally, the biofilm experiments demonstrate that competition can actually strengthen cooperation, if phenotypic traits within a population diversify under selective pressure. The biofilm experiments provide compelling evidence for character displacement as a means by which cooperating—at the very least, co-existing—species may become more productive and less susceptible to invasion by cheats.

Collectively, the experiments show that cooperation is far more robust and the mechanisms for maintaining it more varied than previously thought. It is unlikely that abstract models and computer simulations could have revealed this. Abstract models are based on the current state of knowledge, which is only driven forward by nature put to the test in ecologically plausible conditions, often with unexpected results.

Wilson and Hölldobler (2005) note that the “breakthrough” to eusociality conferred “spectacular ecological success” on those species in which it occurred, especially the ants and termites, which together account for more than half the planet’s total insect biomass while comprising “only 2%” of the known insect species (p. 13370). Yet Wilson and Hölldobler claim that the conditions favouring development of eusociality are extremely rare. Can this really be so? Even more than do eusocial insects, bacteria enjoy a degree of ecological success that is awe-inspiring. Bacteria have colonized every conceivable niche on Earth, from the clouds to the depths of the planet’s crust, and indeed almost every other living thing upon it—including eusocial insects. In *Homo sapiens*, resident bacteria are estimated to outnumber the cells of the human body. It does not seem too wildly speculative to presume that the unsurpassed ecological success of bacteria has something to do with their cooperative behaviour, and that their cooperative behaviour may have evolved roughly along the lines of the most successful multicellular taxa, as well as along lines we have yet to identify. The main message of the microbiological revolution of the past two decades is that bacteria are surprisingly complex creatures, with much still to teach about many biological, behavioural, ecological and evolutionary processes.

If cooperative behaviour is indeed common in the microbial world, then the conditions that give rise to cooperation—including extreme forms of self-sacrifice—are not rare. If fossil stromatolites were indeed formed by ancient microbes acting together, as recently suggested, cooperation may also be an evolutionarily ancient strategy. If cooperation is widespread and ancient, then it cannot be puzzling. Accordingly, some of the assumptions about how natural selection works, which have led to the "enduring paradox of collateral altruistic behaviour", require modification. Recent experience suggests that bacterial models will help provide the means for testing those assumptions and provide some, perhaps many, of the insights necessary for shaping the evolutionary theory of the future.

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[1] Indirect reciprocity and by-product mutualism are often listed as the same phenomenon. However, some authors (e.g., Nowak 2006) identify 'indirect reciprocity' as a form of cooperation based on reputation, which is different from by-mutualism, so I have separated them here.

[2] .A class-structured population is one in which phenotypically distinct types (e.g., reproductives, workers, sentinels) perform different tasks within the group, such as in ant colonies.

[3] Archaea have not been used in studies concerning cooperation, quorum sensing or biofilm formation, to my knowledge, so the discussion here is limited to bacteria.

[4] Unlike many other Gram-negative bacteria that form complex multicellular aggregates when nutrients are available, *M. xanthus* forms fruiting bodies only under starvation conditions (O'Toole et al. 2000).

[5] Redfield (2002) claims the common assumption that quorum sensing is a form of communication is mistaken on the basis that kin selection is the only possible basis for the evolution of bacterial cooperation, and kin selection-based sociality does not require communication; limited disperal of offspring is enough. Therefore, neither "the [evolutionary] need for group action nor the selective conditions required for its evolution" has been demonstrated in bacteria (p. 365). Quorum sensing could be a means for detecting "the physical structure of the environment"—specifically, the diffusion properties of the surrounding medium—"rather than the presence of other bacteria" (p. 365, 368). However, as the siderophore experiments show, cooperation can develop among bacteria even when genetic relatedness is low, which is consistent with the Wilson-Hölldobler model that gives kin selection a secondary role in the development of extreme cooperation. Kin selection theory cannot explain the predominance of multispecies bioflims in nature, either, yet the survival benefits of 'group action' in these pervasive consortia have been amply demonstrated. Redfield's scepticism also now stands against a voluminous, expanding literature. Keyword searches on PubMed (25-12-06) displayed the following references: 'quorum sensing' (1348); 'bacteria' and 'biofilms' (5579); 'quorum sensing' and 'biofilms' (234); 'bacteria' and 'conjugation' and 'quorum sensing' (37).

[6] This is perhaps understandable. Quorum sensing is often referred to as 'language' (Bassler 2002; Sperandio et al. 2003), 'talking' (Kaiser 1993; Winzer et al. 2002), 'listening' (Fuqua and Greenberg 1998), 'eavesdropping' (von Bodman et al. 2003), and even 'linguistic communication' (Ben-Jacob et al. 2004)—sometimes without scare-quotes. Adopting a principle of charity, the tendency of many QS

researchers to use language associated with human communication can be viewed as principally pragmatic or heuristic. A potentially more serious challenge, however, is Keller and Surette's distinction between 'signals', which are evolved to carry information between a sender and receiver, and mere 'cues', a term they imply covers most autoinducers (Keller and Surette 2005, p.253). Leaving aside the difficulty of disentangling a trait that is 'evolved for' some function and one that performs the function due to exaptation, autoinducers meet widely accepted criteria for information-bearing entities. They are molecules produced by an organism to gather information about the world that have meaning solely by virtue of an evolved coadaptation between sender and recipient. In contrast to nutrients, oxygen, light, etc., there is nothing intrinsically informative or indicative about autoinducers, and only in particular contexts do they bear any meaningful information at all. Thus they meet Maynard Smith's key criterion for 'biological information', which is a degree of "arbitrariness" between signal and signified (Maynard Smith 2000). Autoinducers also fit the concept of "intentional signs" (Millikan 2004) with "primitive content" (Harms 2004) in the terminology of teleofunctional semantics.

[7] Bacteriocins are chemical weapons bacteria deploy against other microorganisms. They are often referred to as antibiotics.

[8] This definition of intracellular signalling combines elements of Shimkets (1999) and Watnick and Kolter (2000).

[9] Overall mechanism does not refer to structure here but to the way something works (Machamer et al. 2000).

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