13

Social behaviour in microorganisms

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OVERVIEW

Sociobiology has come a long way. We now have a solid base of evolutionary theory supported by a myriad of empirical tests. It is perhaps less appreciated, however, that first discussions of social behaviour and evolution in Darwin's day drew upon single-celled organisms. Since then, microbes have received short shrift, and their full spectrum of sociality has only recently come to light. Almost everything that a microorganism does has social consequences; simply dividing can consume another's resources. Microbes also secrete a wide range of products that affect others, including digestive enzymes, toxins, molecules for communication and DNA that allows genes to mix both within and among species. Many species do all of this in surface-attached communities, known as biofilms, in which the diversity of species and interactions reaches bewildering heights. Grouping can even involve differentiation and development, as in the spectacular multicellular escape responses of slime moulds and myxobacteria. Like any society, however, microbes face conflict, and most groups will involve instances of both cooperation and competition among their members. And, as in any society, microbial conflicts are mediated by three key processes: constraints on rebellion, coercion that enforces compliance, and kinship whereby cells direct altruistic aid towards clonemates.

13.1 Introduction

> We must be prepared to learn some day, from the students of microscopical pond-life, facts of unconscious mutual support, even from the life of micro-organisms.
>
> Kropotkin (1902).

The idea of sociality in the mere microbe can be met with a raised eyebrow and a smirk. Nevertheless, for as long as there has been evolutionary biology, and indeed sociology, microbes have featured in descriptions of social life. Prominent among these are the writings of Herbert Spencer, the social philosopher who coined the term *survival of the fittest* in the wake of Darwin's *Origin*. Spencer was widely responsible for popularising the notion of altruism in Victorian Britain (Auguste Comte probably first coined the term), and importantly used both humans and single-celled life to define altruism's nature (Dixon 2008).

Social Behaviour: Genes, Ecology and Evolution, ed. Tamás Székely, Allen J. Moore and Jan Komdeur. Published by Cambridge University Press.

And it was not long before a near-modern perspective emerged at the hands of the eccentric Russian explorer and anarchist Peter Kropotkin. In what was arguably the first sociobiology text, Kropotkin ran the gamut of examples of biological cooperation. Many were inspired by his wanderings through frozen Siberia, but his imagination wandered further to include speculations on microbial life.

The concepts of altruism and cooperation in biology, therefore, were developed with the appreciation that they might be applied to even the smallest of organisms. From there, more familiar organisms took centre stage in the developing field of ethology (Chapter 1; Tinbergen 1963), which later became sociobiology (Hamilton 1964, Wilson 1975). In the last century the spectacularly social insects, cooperatively breeding vertebrates and, of course, we humans have been widely studied and drawn upon to test the core theories of social behaviour. The colourful chapters that comprise the majority of this book are a testament to this. But there was little word on the microbes. Until, that is, microbiologists started a revolution from within and challenged the oft prevailing view that the microorganism is a self-absorbed creature, swimming alone in the plankton. Researchers were finding that many species attach to surfaces, secrete structural polymers, and grow into large and sometimes diverse communities (Fig. 13.1; biofilms: Kolter

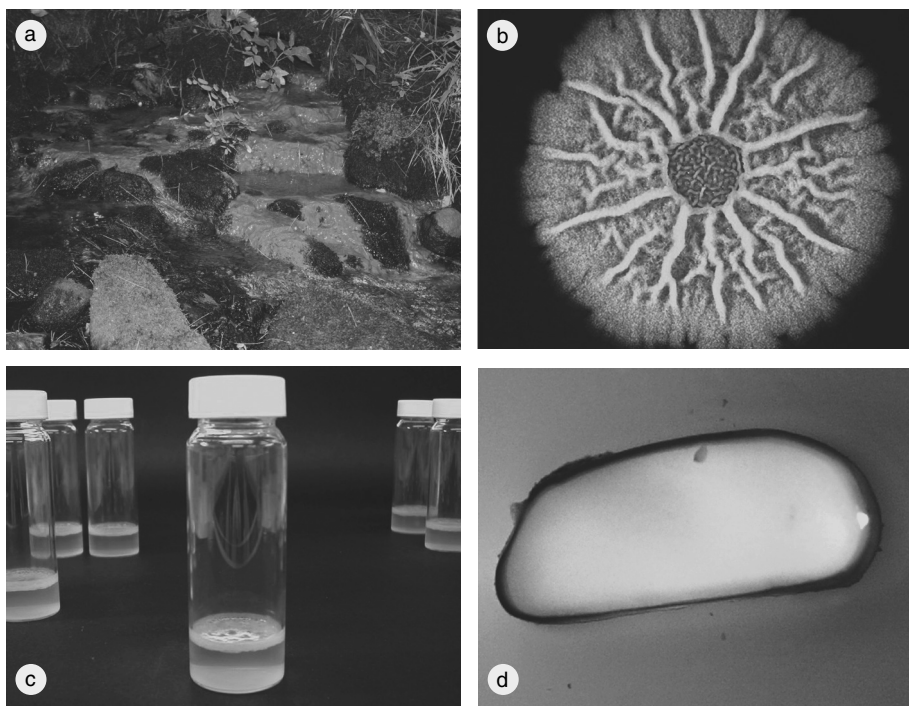

Figure 13.1 Microbial groups. (a) Large microbial biofilm in a Massachusetts river, USA. (b) Colony of the spore-forming bacteria *Bacillus subtilis* growing on agar. The cells secrete structural polymers that contribute to the wrinkly appearance. (c) Biofilm at the air–water interface in the bacterium *Pseudomonas fluorescens*, again formed with the help of extracellular polymer secretion. (d) Flocculation behaviour in the budding yeast *Saccharomyces cerevisiae*. A slice through a yeast 'floc' is shown, which forms after the yeast cells have aggregated (Smukalla *et al*. 2008). The floc has been treated with a toxin (ethanol). The dark area shows dead cells on the outside, but these protect the cells on the inside. Photos: (a) by the author, (b) by Hera Vlamakis, (c) by Andrew Spiers, (d) by Kevin Verstrepen.

& Greenberg 2006, Nadell *et al*. 2009). This led to a dramatic shift in perspective. Indeed the pendulum may have swung too far at first, with some descriptions bordering on the utopian: 'biofilms resemble the tissues formed by eukaryotic cells, in their physiological cooperativity and in the extent to which they are protected from variations in bulk phase conditions by a primitive homeostasis provided by the biofilm matrix' (Costerton *et al*. 1995). But with the close of the last century, the study of social behaviour in microorganisms bloomed, and most recently it has come to include sociobiologists, such as myself, who cut their teeth on studies of more classic social organisms (for me, it was the social wasps: Foster & Ratnieks 2001). And the microbes bring a valuable new perspective because, for the first time, we can hope to find the genes that underlie social behaviours and watch the emergent dynamics of social evolution (Foster *et al*. 2007).

But what exactly is a social behaviour in a microorganism? First things first; the use of *microorganism* here will mean a focus on species with individuals that we cannot see unaided and, in particular, the well-studied bacteria (Box 13.1). *Social behaviour* simply means a behaviour that affects another cell's evolutionary fitness (Table 13.1; Hamilton 1964, Wilson 1975), and the most interesting social behaviours are the ones that evolved *because* they affect others (West *et al*. 2007). Only if a behaviour evolved because of its social effects, or at least partly due to them, can it strictly be viewed as a social strategy. Consider a cell that secretes a waste product which harms another cell. One can probably safely assume that the act of secretion is the product of natural selection on the secreting cell, but the harm caused to the other cell may not be; it may simply be a by-product of natural selection to remove waste (Diggle *et al*. 2007a).

Box 13.1 Some key players in microbial social evolution

Dictyostelium discoideum – This slime mould or social amoeba is actually a member of a little-known kingdom, the Mycetozoa, which is the sister group of animals and fungi. *D. discoideum* is a bacterial predator that displays impressive multicellularity, aggregating upon starvation to form a multicellular slug and then a fruiting body, in which some cells die to hold the others aloft as spores (Fig. 13.6).

Bacillus subtilis – A spore-forming soil bacterium that forms tough biofilms on agar and on the surface of growth medium (Fig. 13.1b). These biofilms show considerable differentiation, including isolated areas of spores and other areas containing cells that secrete the polymers that bind the group together. Under other lab conditions, *B. subtilis* cells will also diversify into competent and non-competent cells, and into chaining and single cells (see main text).

Escherichia coli – The classical laboratory species, *E. coli* is found in the soil and, of course, in the digestive tracts of many healthy animals. *E. coli* displays evidence of cooperative entry into a dormant state upon starvation, and can carry a plasmid that poisons cells that do not possess it.

Myxococcus xanthus – A striking example of convergent evolution with *D. discoideum*, this spore-forming soil bacterium will aggregate upon starvation to form a fruiting body. *M. xanthus* cells also secrete toxins that kill other cells within the fruiting body, other strains, and also species upon which it feeds. The latter process is sometimes associated with social motility, an example of group predation (Fig. 13.5a).

Pseudomonas – *P. aeruginosa* is a pathogenic bacterium that is sometimes found in soil and often in the clinic (Fig. 13.2b). While best known for its ability to cause complications in the lungs of cystic fibrosis patients, it can cause problems in any

Box 13.1 Continued

immunosuppressed patient. *P. aeruginosa* also forms robust biofilms (Fig. 13.4), displays quorum sensing, undergoes swarming motility, and secretes many shared products including siderophores that help with the uptake of iron. *P. fluorescens* is a generally non-pathogenic soil bacterium that also forms biofilms, most famously in the form of a mat on the surface of standing cultures in the laboratory (Fig. 13.1c).

Saccharomyces cerevisiae – The workhorse of eukaryotic cell biology, the budding, brewer's or baker's yeast has been cultivated by humans for millennia. While laboratory strains are often grown in a manner that limits social behaviours, this species secretes several enzymes, will aggregate with other cells by flocculation (Fig. 13.1d), and can undergo pseudo-hyphal growth whereby growing cells do not separate after division and form long chains.

Vibrio – *V. cholera* is the bacterium that causes cholera through the colonisation of the human intestine and the subsequent secretion of cholera toxin. Growth in the intestine and in the environment is often associated with biofilm formation and quorum sensing. The related species *V. fisheri* lives mutualistically in the light organ of the bobtail squid and produces light under quorum-sensing regulation.

The heart of this chapter is a review of the social behaviours of microorganisms, and the possible evolutionary benefits they carry to the cells that express them (section 13.2, *Form and function*). They range from the simple effects of growth rate through to complex multicellular development, with secretion, communication and genetic exchange in between. Some behaviours are downright selfish, but others have the appearance of cooperation, whereby the actions of one cell increase the reproductive fitness of another (Table 13.1; West *et al*. 2006). Identifying when behaviours are cooperative versus selfish – or the associated question of when they are a true social strategy versus a by-product of non-social traits – can be difficult for microbes. This is due in no small part to our limited understanding of microbial behaviours in nature. For example, when is it good, evolutionarily speaking, to be in a biofilm? Moreover, what really is a microbial group? A cell may sit in a massive biofilm but only ever affect its very nearest neighbours. So please take all interpretation herein with such caveats and questions in mind.

That said, many behaviours do seem to slow or even prevent a cell's division, which means a reduction in personal fitness. Keeping focus then not on the strain but on the individual cell, these behaviours have the hallmark of microbial altruism (Hamilton 1964, West *et al*. 2007, Foster 2008), and even spite (Foster *et al*. 2001, Gardner *et al*. 2004) (see Table 13.1 for definitions). Finding microbial altruism, we are faced with the classic problem of sociobiology (Hamilton 1964, Wilson 1975): how can apparently selfless traits remain stable in the face of natural selection for conflict and cheating (Chapter 6)? Section 13.3, *Conflict resolution*, is dedicated to this question.

13.2 Form and function

13.2.1 Growth rate

A simple way to affect your neighbours is to steal their food. This is particularly easy when you share resources like carbon and oxygen as a microbe, and when these become growth-limiting the potential for conflict is clear (Ratnieks *et al*. 2006). Natural selection can favour cells that hungrily consume resources in order to obtain the lion's share, even though this may be inefficient and give poor future prospects (Kerr *et al*. 2006). At least mathematically, this is analogous to the ongoing clashes over fisheries, and air quality between laissez-faire capitalists on the one hand and environment-oriented interventionists on the other (the tragedy of the commons: Chapter 6, Rankin *et al*.

Table 13.1. Definitions of social behaviour based upon the effects on personal lifetime reproduction (direct fitness)

		Effect on *recipient*	
		Positive	Negative
Effect on *actor*	Positive	Mutual benefit	Selfishness
	Negative	Altruism	Spite
		Cooperation	***Competition/conflict***

2007). Many microbes have the ability to switch from aerobic respiration to fermentation, which produces energy more rapidly when oxygen is limited. However, fermentation is also inefficient and yields much less energy overall (Pfeiffer *et al*. 2001, Pfeiffer & Schuster 2005, Novak *et al*. 2006). Competing cells therefore are expected to more often make use of fermentation (MacLean & Gudelj 2006), while cooperative systems, including multicellular organisms, are more likely to be altruistically aerobic, whereby each cell slows its growth to allow other cells to have food in the future (Kreft 2004, Kreft & Bonhoeffer 2005).

High growth rate has many potential knock-on effects. One much discussed is the evolution of virulence, where rapid pathogen proliferation can mean trouble for a patient (Frank 1992). However, dividing rapidly will also tend to take resources away from cooperative traits like product secretion (Griffin *et al*. 2004), communication (Sandoz *et al*. 2007) and development (Velicer *et al*. 1998, Vulic & Kolter 2001); these are discussed below. When these systems are required for microbes to successfully attack the host, rapid growth can again lower ultimate yield, and lead to reduced, rather than increased, virulence (Brown *et al*. 2002, Foster 2005, Harrison *et al*. 2006).

13.2.2 Secreted products

Microbes secrete all manner of substances that physically and chemically alter their surroundings and their evolutionary fitness (Fig. 13.2). There are several ways out of a cell, including passive diffusion (Pearson *et al*. 1999), active pumps (Kostakioti *et al*. 2005), packaged in mini-membranes (vesicles: Mashburn-Warren & Whiteley 2006) and of course when a cell bursts (Cascales *et al*. 2007). By secreting products, cells can create an environment that promotes growth – a simple form of niche construction (Lehmann 2006). For example, when cells of the budding yeast *Saccharomyces cerevisiae* get low on glucose, they secrete an enzyme called invertase that hydrolyses sucrose to liberate glucose and fructose (Greig & Travisano 2004).

Other secreted products bind and scavenge nutrients. The pathogen *Pseudomonas aeruginosa* is a fascinating but unpleasant bacterium that infects the lungs of patients with cystic fibrosis; a genetic disorder causing, amongst other symptoms, thickened airway mucus. *P. aeruginosa* relies on many secreted products for infection including siderophores (literally 'iron carriers' in Greek; Fig. 13.2). These dissolve insoluble iron and allow the bacterium to combat a common vertebrate response known as localised anaemia that reduces the iron in tissue (Jurado 1997). But the conflict lies not only with the patient. There is also the potential for infighting: mutants that neglect secretion are able to cheat those that do (Griffin *et al*. 2004), and the emergence of cheating can lead to reduced virulence in animal models (Harrison *et al*. 2006). It is interesting that the genetics underlying both yeast invertase and the siderophores display high variability (Greig & Travisano 2004, Smith *et al*. 2005). This may indicate shifting ecological demands (Brockhurst *et al*. 2007, Foster & Xavier 2007), or even an evolutionary arms race in which cooperators attempt to evolve products that cannot be used by cheaters (Tuemmler & Cornelis 2005).

But it is not all darkness and disease. Many microbes provide metabolic assistance by producing extracellular compounds or resources that their host

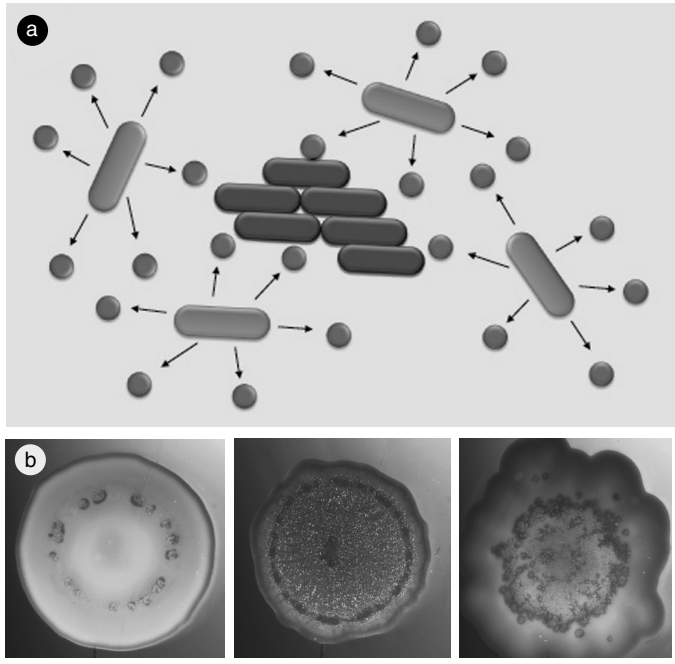

Figure 13.2 Secreted products in microbes. (a) Cooperative secreted products and cheating: the paler cells secrete a product that benefits all cells, but the darker cells do not, and save energy in the process. The darker cells cheat and outgrow the paler cells. (b) Different strains of the bacterium *Pseudomonas aeruginosa* growing on agar displaying different pigmentations that result, at least in part, from differences in pyoverdin (green, left) and pyocyanin (blue, middle) secretion, which both function in iron scavenging. The far-right strain also shows the characteristic spotting of viral-driven cell-lysis. Photos by the author.

organisms cannot make. This includes our own gut fauna, and also antifungal bacteria that protect leaf-cutter ant fungal gardens (Currie *et al*. 1999), light-producing *Vibrio fisheri* in fish and squid (Visick *et al*. 2000), and nitrogen-fixing bacteria or mycorrhizal fungi (Helgason & Fitter 2005) that provide nutrients for plants (Denison 2000). Cross-feeding among the microbes themselves also appears common, whereby the waste product of one species feeds another (Dejonghe *et al*. 2003), which, in turn, keeps the waste product at low levels and may improve their combined metabolism (Pfeiffer & Bonhoeffer 2004). With many species interacting, there is the potential for cross-feeding networks to reach dizzying complexity. One nice example occurs inside an insect, itself an exemplar of animal sociality: a termite. Termite society rests upon the ability to eat wood, and they are helped along by protozoa that break down cellulose (Fig. 13.3; Noda *et al*. 2003). These protozoa in turn rely on bacteria, often spirochetes, that provide both metabolic assistance (Dolan 2001) and even motility (Tamm 1982, Wenzel *et al*. 2003, König *et al*. 2007). Meanwhile, some spirochetes rely on cross-feeding from yet other bacteria in the termite gut (Graber and Breznak 2005).

Shared products can also be structural. While not strictly secreted, the RNA virus φ6 produces proteins using the cellular machinery of its bacterial host. These proteins package the viral RNA in a protective coat and can be used by all viral RNAs such that cheater virus RNAs, which do not encode the proteins, can still be successfully packaged (Turner & Chao 1999, Brown 2001). While viruses coat themselves in rigid protein coats, bacteria instead use slime. More formally known as extracellular polymeric substances (EPS), the slimes found in bacterial biofilms

Figure 13.3 The symbioses inside termites, which feed on wood. (a) The host: the termite *Mastotermes darwiniensis*. (b) A symbiont, *Mixotricha paradoxa*, that helps to break down cellulose. The fur on the surface is actually the flagella of symbiotic spirochete bacteria that help it to swim around (König *et al*. 2007). Photos: (a) by Judith Korb, (b) by Helmut König.

are complex mixtures that probably protect the cells from various stresses (Crespi 2001, Velicer 2003, Hall-Stoodley *et al*. 2004, Foster 2005, Keller & Surette 2006, West *et al*. 2006). Slime may even allow one strain to smother and suffocate others in the quest for oxygen and nutrients, in the manner that a tree trunk allows one plant to grow tall and shade another (Fig. 13.4; Foster & Xavier 2007, Xavier & Foster 2007).

Although sinister, a bit of smothering is nothing compared to the favoured weapon in the microbial arsenal: secreted toxins. The toxin–antitoxin systems carried by many bacteria are probably the closest thing that microbes have to aggression (Chapter 7). In their simplest form, these systems comprise two neighbouring genes, one encoding a toxin (bacteriocin) that kills other strains, and the other an antitoxin, or immunity protein, that protects the toxin-producing strain. The cystic fibrosis pathogen *P. aeruginosa* carries multiple such toxin secretion systems, which evolve rapidly and may again be indicative of arms races (Smith *et al*. 2005). If *P. aeruginosa* is indeed involved in an arms race with other species, it appears to do well in the rankings: we find it near impossible to get accidental contamination of *P. aeruginosa* cultures in our laboratory. Another interesting toxin–antitoxin system is found on a plasmid (a small, circular, extrachromosomal piece of DNA) carried by the well-known bacterium *Escherichia coli*. The toxin appears only to be released when some plasmid carriers burst, taking non-carrier cells down with them in dramatic fashion (Cascales *et al*. 2007), an example of evolutionary spite (Gardner *et al*. 2004). But it does not always end there, because the success of toxin-producing cells paves the way for a second strain that produces only the antitoxin and saves on the cost of the toxin. Then this second strain can be replaced by the original susceptible cell type that produces neither toxin nor antitoxin (Kerr *et al*. 2002), which means that toxin producers can reinvade, and so on. This non-transitive (rock–paper–scissors) relation can lead to cyclical dynamics that maintain all three types, and which may represent a general principle for the maintenance of biodiversity (Durrett & Levin 1997, Kerr *et al*. 2002, Reichenbach *et al*. 2007).

It is tempting to think that toxin–antitoxin systems always go hand in hand with conflict. Perhaps not; several examples have been instead interpreted in terms of cooperation, like that seen between the

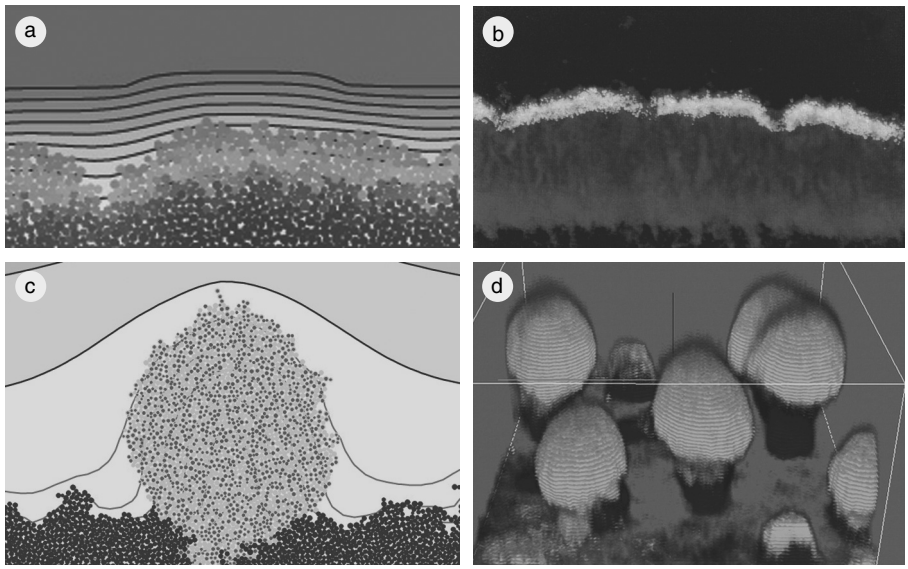

Figure 13.4 Nutrient competition and motility in bacterial biofilms. (a) An individual-based model of a microbial biofilm. The cells respire and in doing so create oxygen gradients (parallel lines) that slow the growth of cells deep in the biofilm (the paler cells are growing quickly and the darker ones slowly). (b) A biofilm of *P. aeruginosa* in which metabolically active cells have been stained yellow (the palest part of the image). (c) The effect of polymer secretion on success within a biofilm. The simulation shows cells that secrete a polymer and altruistically push their descendants up into the oxygen, which suffocates the cells beneath (shown dark) that do not secrete (Xavier & Foster 2007). (d) The effect of motility on success within a biofilm. Confocal image of a *P. aeruginosa* biofilm with a motile strain (pale) that moves towards the top of the biofilm and a non-motile strain (dark) that does not (Klausen *et al*. 2003). Images (a) and (c) from Joao Xavier, (b) from Phil Stewart, (d) from Tim Tolker-Nielsen.

germ and soma cells of your body. The *E. coli* chromosome contains the *mazEF* system (Metzger *et al*. 1988), where *E* encodes the antitoxin and *F* the toxin. This system has a clever feature: the two genes turn on and off together (they are an operon) but the toxin degrades more slowly than the antitoxin. This means that when the *mazEF* operon is deactivated, the levels of toxin and antitoxin in the cell will steadily decrease but, importantly, the antitoxin disappears first, leaving behind the toxin, and this kills the cell. What makes this clever is that various environmental stresses turn off the operon, including heat, DNA damage and virus attack, so that damaged cells will undergo lysis (Hazan *et al*. 2003). The process looks a lot like an altruistic behaviour – the weakened committing suicide to protect the strong – and there is evidence that *mazEF*-induced lysis of diseased cells can inhibit the spread of viruses to healthy cells (Hazan and Engelberg-Kulka 2004). However, the benefits under other stresses are less clear (Tsilibaris *et al*. 2007), and more work is needed to dissect out the exact evolutionary pressures that drive the *mazEF* system and other toxin systems (Magnuson 2007). Critically, if lysis releases the toxin and kills non-carriers, like the colicins, then *mazEF* may also function in competition with other strains and species. Similar questions await the spore-forming bacterium *Bacillus subtilis* (Fig. 13.1b), which actively secretes a toxin that kills non-expressing cells in the surroundings (Gonzalez-Pastor *et al*. 2003, Dubnau & Losick 2006), and viral-induced cell death in *P. aeruginosa* biofilms (Fig. 13.2b; Allesen-Holm *et al*. 2006), which have both been interpreted as purely cooperative behaviours.

13.2.3 Communication

Microbes also use secretions to communicate with each other. Indeed, killing another cell with a toxin is a very simple, albeit rude, form of discourse. However, this would not, strictly speaking, be communication (or signalling) by some evolutionary definitions (Chapter 8), which demand that both producer and receiver benefit from their respective actions (Keller & Surette 2006, Diggle *et al*. 2007a). Such cooperative communication does seem likely in some systems, including the coordinated development of slime moulds discussed below, and perhaps the curious tendency of *S. cerevisiae* yeast cells to synchronise their intracellular physiologies, even though cell division is not synchronised (Tsuchiya *et al*. 2007). Microbes also display an impressive ability to detect the density of their own and other species through quorum sensing (literally, sensing who is around: Fuqua *et al*. 1994). Quorum sensing is found both in bacteria (Miller & Bassler 2001, Keller & Surette 2006, Diggle *et al*. 2007a) and in fungi (Hogan 2006), and involves a wide variety of secreted compounds known as autoinducers, including some packaged in vesicles (Mashburn & Whiteley 2005). All exploit the same simple principle: if cells secrete a chemical, then as cell density goes up, so will the local concentration of the chemical. By responding to the chemical, therefore, the cells can respond to their own density.

Quorum sensing was first characterised in *V. fisheri*, the glow-in-the-dark bacterium that lives in the sea, where it exists in a free-living form but also on and in squid and fish, whom it helps with behaviours like mate or prey attraction (Nealson *et al*. 1970, Fuqua *et al*. 1994). *V. fisheri* regulates luminescence with an autoinducer, an *N*-acylhomoserine lactone, such that the cells only turn on at high density in the host, and not when swimming in the sea. Moreover, this molecule works through a positive-feedback loop whereby it induces an increase in its own production. Since then, the name has spread to quorum-sensing molecules that do not autoinduce, and quorum sensing has been found to control a multitude of behaviours, including bacterial spore formation (Hagen *et al*. 1978, Gonzalez-Pastor *et al*. 2003, Dubnau & Losick 2006), biofilm formation (Davies *et al*. 1998), the transition from a single-celled to a hyphal lifestyle in fungi (Hornby *et al*. 2001), DNA uptake and exchange (Piper *et al*. 1993, Magnuson *et al*. 1994, Havarstein *et al*. 1995) and many secreted products such as slime (EPS) (Hammer and Bassler 2003), iron-scavenging molecules (Stintzi *et al*. 1998) and toxins (van der Ploeg 2005).

Does quorum sensing always represent active communication from one cell to another? This is not yet clear. Some autoinducers may simply be waste products that did not evolve to allow secreting cells to communicate (Diggle *et al*. 2007a). In some cases, however, autoinducers have been shown to cost energy, which suggests that they do represent active and evolved communication. The cost also means that mute cells, which do not produce the signal, can cheat by listening in without paying the cost of communication, just as cells can cheat on a shared enzyme (Fig. 13.2a, Brown & Johnstone 2001, Diggle *et al*. 2007b). Other times it can pay to play deaf. Some *P. aeruginosa* strains from cystic fibrosis (CF) patients lack a functional copy of the autoinducer sensor protein LasR, which is required to respond to the autoinducer. Lacking the sensor protein stops cells producing cooperative products that may not be needed in the CF lung and, even if the products are needed, the *lasR* mutants can exploit the products of others (D'Argenio *et al*. 2007, Sandoz *et al*. 2007). Finally, strains unable to quorum sense in *V. cholerae* overproduce slime (EPS), which is thought to inhibit dispersal from biofilms but can provide a competitive advantage when it is better to stay put (Hammer & Bassler 2003, Nadell *et al*. 2008).

There is also the potential for communication among species. One quorum-sensing molecule, autoinducer-2 (AI-2), is produced by an amazingly diverse set of species, which opens the possibility of widespread interspecies communication (Federle & Bassler 2003). How often this represents active signalling on the part of secreting cells is not known (Keller & Surette 2006, Diggle *et al*. 2007a), but some species, including *P. aeruginosa*, respond to AI-2 even though they do not make it, which is at least consistent with the evolution of eavesdropping (Duan *et al*. 2003) (Chapter 8). When multiple species meet, there is also the possibility for devious manipulations (Keller

& Surette 2006): *E. coli* actively takes up AI-2, and thereby prevents *Vibrio cholerae* from secreting proteases that it uses during cholera gut infection (Xavier & Bassler 2005). Meanwhile, the dental bacterium *Veillonella atypica* secretes a compound that induces *Streptococcus gordonii* to release lactic acid, which *V. atypica* then feeds on (Egland *et al*. 2004).

13.2.4 Genetic exchange

The social behaviours of microorganisms include sex (Chapter 10). Indeed, eukaryotes like yeast are sexual creatures that undergo meiosis and mating to make recombinant progeny (Goddard *et al*. 2005). Although primarily asexual, bacterial cells also perform more limited DNA exchanges (Thomas & Nielsen 2005, Narra & Ochman 2006) via the environment, viruses and plasmids. A cell able to incorporate DNA from the environment into its genome is termed naturally competent (artificial competence means they will do it when forced by a persuasive lab scientist armed with electrodes or chemicals). Sometimes the act of DNA uptake itself may not be all that social; the cell performing the action may not affect the fitness of others. However, natural competence is often tightly associated with social traits.

V. cholerae becomes naturally competent when growing in a biofilm on arthropod exoskeletons (Meibom *et al*. 2005) and competence in *B. subtilis* involves both quorum-sensing (Magnuson *et al*. 1994) and differentiation (below). Perhaps the most interesting case, however, comes from *Streptococcus* species, which upon entering a competent state also activate multiple toxin–antitoxin systems (above) that selectively lyse surrounding cells that are not competent (Claverys *et al*. 2007). The evolution of DNA uptake is therefore intertwined with potential conflict among strains: the killing factors may both remove competing cells from the environment and also provide DNA for recombination. This adaptive story makes a lot of sense, but at the same time it has a curiously paradoxical aspect to it. Why kill another cell only to incorporate its DNA into your own and partially become it? The answer may lie in recognising that there can be differing evolutionary interests at different loci, and some may favour killing-associated competence, while those that get replaced do not. A human analogy would be the occasions where a conquering nation incorporates some aspects of a subordinate's culture into its own, such as the Romans adopting Greek gods.

Transport of DNA between cells is even more directed when driven by plasmids (conjugation) and viruses (transduction). Plasmids are the relatively small circular pieces of DNA carried by bacteria. Despite their small size, plasmids exert a powerful influence on cell biology and are often able to engineer their own propagation, by inducing their cell to attach to another cell and make a channel through which a plasmid copy can pass (Thomas & Nielsen 2005). Many plasmids are benevolent and produce beneficial proteins, including antibiotic resistance genes, but others act like viruses, and spread within and among cells at a cost to their hosts (Eberhard 1990, Smith 2001, Paulsson 2002). And while plasmids show some specificity, they are often able to transfer their DNA to other species. One example is the tumour-inducing (TI) plasmid that makes the bacterium *Agrobacterium tumefaciens* into a plant pathogen. This plasmid has amazing abilities. It integrates fragments of its DNA into the plant's genome and induces the formation of a gall, which protects and feeds the bacteria. The plasmid also encodes its own quorum-sensing system, and at high quorum it will induce conjugation and transfer itself to other *A. tumefaciens* cells (Chilton *et al*. 1980, Piper *et al*. 1993). Transfers of viral and plasmid DNA between species, however, are not perfect and sometimes take host DNA along for the ride. This can have unintended but important consequences, such as mixing the DNA of prokaryotes and eukaryotes, which are otherwise separated by over a billion years of evolution (Gogarten & Townsend 2005, Thomas & Nielsen 2005).

13.2.5 Group formation

Social interactions are most intense when individuals live side by side in a group (Chapter 9), which for many microbes will mean a biofilm. Biofilms form when cells grow on a surface or attach to each other

to form a living mat (Fig. 13.1) in which cells secrete all manner of products, including the slimy polymers that form a structural matrix around the cells (Fig. 13.4; Branda *et al*. 2005, Parsek & Greenberg 2005, Nadell *et al*. 2009). Interestingly, some strains turn on the slime at high density while others turn it off. Turning on at high density makes sense: slime will only be made in the biofilm and not when swimming around at low density, where slime presumably does not pay. But why do some species then turn it off again at even higher density? The answer may lie in dispersal. Negative regulation has been found in *V. cholerae*, which induces a short-lived acute infection that climaxes with dispersal en masse from the gut of the unfortunate patient. This need to disperse may favour strains that turn off the slime just before they leave, when they will no longer need it (Hammer & Bassler 2003, Nadell *et al*. 2008). Biofilms often also contain many species. *V. cholerae* must compete in the gut with many other species including *E. coli*, which can interfere with its quorum sensing, as we have seen (Xavier & Bassler 2005). The best studied multispecies biofilms, however, must be those growing upon your teeth as you read this. Dental plaque contains an amazing diversity of species (Kolenbrander 2000), including some that depend upon each other for attachment and growth (Palmer *et al*. 2001).

Living in a dense biofilm presents challenges, including intense nutrient competition that threatens to slow growth (Fig. 13.4a, b; Stewart 2003, Xavier & Foster 2007). So why make one? The answer is likely to vary between species (Hall-Stoodley & Stoodley 2005), but one common benefit is probably settling in a good place to live. A biofilm at an air–water interface has good access to oxygen and light (Fig. 13.1c; Rainey & Rainey 2003, Brockhurst *et al*. 2007), and attachment to solid surfaces can yield similar advantages, particularly given that cells will often attach reversibly and swim off if they end up in a bad spot (Sauer *et al*. 2002). Biofilm life also allows cells to condition the local environment to suit growth and communication (Parsek & Greenberg 2005, Keller & Surette 2006), and they are tough: biofilms are the scourge of industry and medicine, where they clog, contaminate and cake machines and people alike (Fux *et al*. 2005). This suggests that the biofilm state provides considerable protection to its inhabitants, which can include resistance to antibiotics (Mah *et al*. 2003). Analogously, it has recently been shown in the yeast *S. cerevisiae* that flocculation, whereby cells aggregate together at high densities, provides protection against stresses and antifungal agents (Fig. 13.1d; Smukalla *et al*. 2008).

Bacterial biofilms can also be dynamic environments as strains struggle to get the best nutrients, both by altruistically pushing descendant cells with slime (above, Fig. 13.4c; Xavier & Foster 2007) and by active cell movement (Fig. 13.4d; Klausen *et al*. 2003). At its height, bacterial movement borders on mass exodus when dividing cells engage in a group migration away from high-density areas, in a process known as swarming. In nature, swarming may often be associated with dispersal from biofilms, and, like biofilm formation, swarming is often controlled by quorum sensing (Daniels *et al*. 2004): an exodus is best when it is really crowded. Furthermore, the cells appear to cooperate with one another to secrete products that ensure things go swimmingly, including digestive enzymes (Bindel Connelly *et al*. 2004) and chemicals that ease movement by reducing surface tension (biosurfactants: Kohler *et al*. 2000). In swarming *sensu stricto*, cells propel themselves with beating flagella (Harshey 2003), but they achieve similar ends by pulling themselves along with pili (the molecular equivalent of grappling hooks), and perhaps even by jetting slime out the back, jet-ski-like (Kaiser 2007). A single species can mix and match its methods: a strain of *Myxococcus xanthus* that had been mutated to stop pili-based swarming was able to re-evolve motility using a different mechanism (Fig. 13.5a; Velicer & Yu 2003). But not all microbes migrate outwards in this way. Some actively seek each other out and move together, and it is here that we find the pinnacle of microbial sociality, multicellular development.

13.2.6 Differentiation, diversification and development

Not all cells are equal. Many microbes can differentiate into distinct physiological states and morphologies. In the lab, differentiation events can be relatively

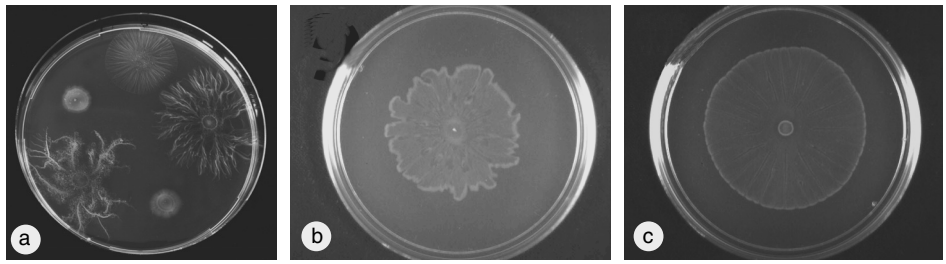

Figure 13.5 Swarming behaviours in bacteria. (a) Different swarming genotypes in the spore-forming bacterium *Myxococcus xanthus* (Velicer & Yu 2003). (b) Swarming cells in *Salmonella enterica*, which become elongated and carry many flagella for efficient propulsion (Kim *et al*. 2003). (c) A second strain of *Salmonella enterica* swarming. Photos: (a) by Greg Velicer, (b) and (c) by Wook Kim.

uniform, such as differentiation into swarming cells in *Salmonella enterica*, which become elongated and carry many flagella for efficient propulsion (Fig. 13.5b, c; Kim *et al*. 2003). But this can change in more complex environments. Cells bathed in oxygen at the surface of a biofilm may divide furiously while those below do little more than provide anchoring (Fig. 13.4a, b; Xu *et al*. 1998). Moreover, diversification into multiple cell types can occur without environmental gradients. One champion is *B. subtilis*, which can form both individual cells and chains of cells during growth, divide into competent and non-competent cells, and then form some spore cells that kill the others (above; Dubnau & Losick 2006). Another important example is the small subpopulations of dormant cells in many bacteria groups that are immune to many antibiotics (persister cells: Balaban *et al*. 2004, Keren *et al*. 2004, Gardner *et al*. 2007). Yeast diversify too: recent studies have shown bimodal expression of genes involved in phosphate uptake (Wykoff *et al*. 2007), and high variability in the tendency to become spores (Nachman *et al*. 2007). Finally, we also find microbial differentiation and diversification in symbioses. Some nitrogen-fixing bacteria in plant roots differentiate into swollen and terminal bacteriods that lose the ability to divide (Mergaert *et al*. 2006), while other cells in the surroundings remain viable and may even be altruistically fed by the differentiated cells (Denison 2000).

How do microbial cells diversify without external cues? The secret of course is *internal* variability. Nutritional state is one way to go. Recently divided cells in aggregates of the slime mould *Dictyostelium discoideum* are low on food, and as a consequence end up dead in a stalk rather than becoming reproductive spores (Fig. 13.6; David Queller's profile; Gomer & Firtel 1987). True randomness is another way to go. Entry into competence in *B. subtilis* is driven by a positive-feedback loop; once a cell starts down that road, there is no pulling out. However, entry is not guaranteed and appears to be driven by chance fluctuations in expression of the regulatory genes – the molecular equivalent of rolling dice (Suel *et al*. 2006). Another possibility in eukaryotes such as yeast is random acts of gene silencing by modification of DNA, or the nucleosomes that associate with DNA, which can suppress transcription (Rando & Verstrepen 2007). Or, more drastically, cells can undergo genetic changes to diversify their phenotypes. This appears to occur in *P. aeruginosa* biofilms, where multiple growth phenotypes appear from a single-type, and do so dependent upon *recA*, the gene central to DNA modification and repair (Boles *et al*. 2004). Finally, the tendency of the *S. cerevisiae* cells to stick to each other by flocculation is controlled by a gene with a large repeat sequence that makes flocculation evolve rapidly and reversibly (Fig. 13.1d; Verstrepen *et al*. 2005, Smukalla *et al*. 2008).

Why would a single strain diversify into multiple phenotypes? There is no doubt that some of the variability among cells is a simple by-product of unavoidable noise in cellular processes, and this must be kept in mind. But for cases that represent real evolutionary adaptations, there are two major explanations for

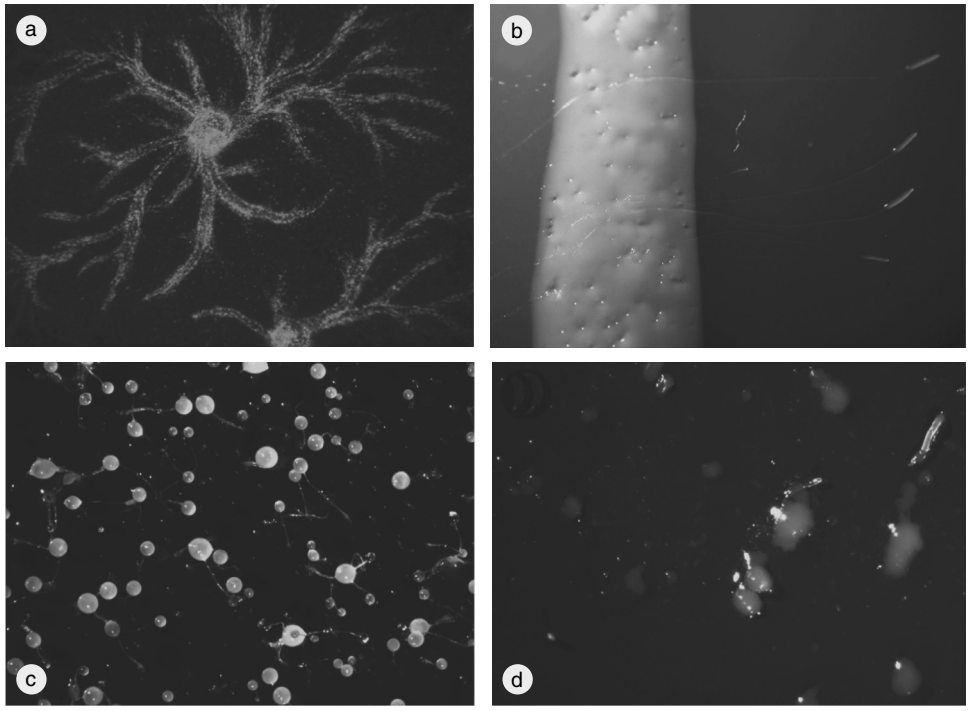

Figure 13.6 Social life, and strife, in the slime mould, or social amoeba, *Dictyostelium discoideum*. (a) Cells labelled with fluorescent beads that are streaming together to form an aggregate, which will then differentiate into a migrating slug. (b) Migrating slugs passing left to right through a strip of bacteria. In some hours, cells that were shed by the slugs will consume the bacteria and make new slugs (Kuzdzal-Fick *et al*. 2007), by which time the original slugs will have changed into fruiting bodies containing a stalk of dead cells that holds the rest up as reproductive spores. (c) Birds-eye view of a lawn of *D. discoideum* fruiting bodies. (d) Development in a *D. discoideum* cheater mutant. Alone, the mutant is unable to sporulate, but when mixed with the wild-type cells the mutant is competitively superior and produces more spores (Ennis *et al*. 2000, Gilbert *et al*. 2007). Photos by the author.

functional diversification. One idea is that cells are hedging their bets on the future (Kussell & Leibler 2005). That is, if there may or may not be an environmental catastrophe on the way, it can pay a strain to have some cells growing and others in a protected survival state as persisters (Balaban *et al*. 2004, Keren *et al*. 2004, Gardner *et al*. 2007). The other major overlapping explanation is a division of labour (Michod 2006). Microbes can divide their labour to perform a task, which might be bet-hedging or indeed something else, such as a division between polymer secretion and spore formation (Vlamakis *et al*. 2008). We, of course, are also living breathing testament to the benefits of a division of labour – both in terms of the cells in your body and in our society.

The division of labour in microbial groups is most clear in development, whereby cells undergo a predictable series of changes over time. Simple development can occur without a division of labour. When short of food, for example, *E. coli* cells simultaneously slow their growth and transition into a dormant spore-like state (Vulic & Kolter 2001). The most convincing examples of development, however, include diversification and a division among cells between reproduction and other functions. This includes *B. subtilis* spore formation, but also some species that have evolved a

dramatic way of getting out of a tight spot. When short of its bacterial prey, cells of the soil-dwelling slime mould *D. discoideum* (Kessin 2001, Shaulsky & Kessin 2007) secrete a signalling molecule (cyclic AMP) that induces the cells to stream together and differentiate into a multicellular slug that migrates towards heat and light (Fig. 13.6a). The slug sheds cells as it goes that colonise nearby patches (Fig. 13.6b; Kuzdzal-Fick *et al*. 2007) and near the soil surface transforms into a fruiting body, in which around a quarter of the cells die in a stalk that holds the other cells aloft as dispersing spores (Fig. 13.6c). Analogously, cells of the bacterium *Myxococcus xanthus*, also a bacterial predator, will aggregate upon food shortage to form a fruiting body containing spores (Julien *et al*. 2000). Again, many cells die in the process (Wireman & Dworkin 1977).

As with all social behaviours in microorganisms, with diversification and development comes potential evolutionary conflict. Indeed, whenever two neighbouring cells have a different phenotype, the chances are that one is doing better reproductively than the other. Natural selection, therefore, can favour cells that become the best type all the time, even though this may mess things up for everyone. Examples are seen in the slime moulds and myxobacteria, where single mutations can produce cheaters that over-produce spores in mixtures with other strains (Fig. 13.6d; Ennis *et al*. 2000, Velicer *et al*. 2000, Santorelli *et al*. 2008). But it also applies to the more simple cases, like the evolution of persister cells, which are dormant cells that suffer poor reproductive prospects, at least in the short term (Gardner *et al*. 2007). However, the fact that slime moulds still fruit and persisters persist tells us that disruptive mutants do not always win out. Why not? That comes next.

13.3 Conflict resolution

The social behaviours of microorganisms carry a variety of benefits, from regulating growth to maximise yield, through niche construction and communication, to the formation of dense protective groupings. Although the ability to cash in on these benefits will ultimately depend upon ecological conditions (Brockhurst *et al*. 2007, Foster & Xavier 2007), broadly speaking these are the benefits that favour social life. But, as we have seen, group-level benefits alone may not be enough, and the potential for conflict looms large in the microbial world. But it is not all-out war, and mechanisms that limit conflicts must and do exist. The next sections review these mechanisms, dividing them up into *direct effects* – effects on the reproduction of the cell that performs the social action – and *indirect effects* – effects on cells that share genes with the focal cell (the structure is based upon Ratnieks *et al*. 2006, Gardner & Foster 2008 and references therein).

13.3.1 Direct effects: constraints and coercion

Non-enforced

As for herding wildebeest, if it is good to be in a group, then joining with other cells can simultaneously benefit the joiner and the joinees, which may mean limited conflict (Chapter 9). Increasing the size of a microbial group may often in this way confer benefits on all inhabitants: a large biofilm can provide better protection from the environment, and larger slime-mould slugs migrate further (Foster *et al*. 2002). Once in a group, though, there is the potential for things to turn nasty – a slime-mould cell may well benefit from other cells joining the slug (Fig. 13.6b), but not if they then force it to make a stalk (Santorelli *et al*. 2008). Mechanisms such as enforcement or genetic relatedness may then be needed to keep the peace (below). However, there are also reasons to believe that microbial groups, and indeed all social species, will tend to have intrinsic properties that moderate conflicts (Travisano & Velicer 2004).

If cooperative systems that avoid cheating persist longer than those that do not, over time there will be enrichment for systems with pre-existing constraints on the origin of cheaters (Foster *et al*. 2007, Rankin *et al*. 2007a). There are several mechanisms that might cause these constraints, including reduced mutation rate (below; Harrison & Buckling 2005, 2007) and genetic redundancy, whereby a cooperative trait

is expressed by multiple genes (Foster *et al*. 2007). Another way is through the ubiquitous phenomenon of pleiotropy (each gene affecting multiple traits: Foster *et al*. 2004). For example, the group-wide entry into a dormant spore-like state in starving *E. coli* cultures (above) is sometime hijacked by the GASP mutant (growth at stationary phase: Vulic & Kolter 2001). Like a cancer, the mutant cells keep on growing under low nutrient conditions and replace the normal cells. However, this comes at a pleiotropic cost: long term the cheaters are compromised and probably doomed owing to poor acid tolerance, which prevents the cooperative trait being lost.

The gradual enrichment of constraints on cheater mutations, through pleiotropy or otherwise, may also be driven by shorter-term processes than trait or species persistence. As we will see below, some bacterial strains always have some cells that mutate into cheaters. When this harms the strain's prospects for founding new biofilms, selection will favour the evolution of genetic protection against cheater mutations, in the same way that multicellular organisms gain constraints on cancer mutations (Nunney 1999, Michod & Roze 2001). In this vein, natural selection for cooperation has been shown to lead to reduced global mutation rate in *P. aeruginosa* (below; Harrison & Buckling 2007). Constraints on cheating can also arise through an arms-race-like process in which a cheater strain paves the way for a new strain that can resist the cheater and others like it (Foster 2006). An experiment that mixed cheater strains with natural strains in the spore-forming bacterium *M. xanthus* caused some lab populations to go extinct (Fiegna *et al*. 2006). The cheater strains would rise to dominance, but this drove their own demise because they are impotent without the natural strains. However, in one case, a new mutant arose that could both outcompete the cheaters and form spores on its own: the system had evolved to constrain the cheaters.

Another process that can align evolutionary interests within a social group is niche separation. When strains or species compete, natural selection can favour individuals that specialise on a different resource than the competing species. This can have two knock-on effects. First, diversification may create a saturated environment where all niches are filled, which makes it more difficult for cheaters to arise, as has been seen in bacterial biofilms (Brockhurst *et al*. 2006). In addition, if two species no longer compete, the way is open for evolution of between-species cooperation. This occurs when species can exchange resources that are cheap for the donor but expensive for the recipient (Schwartz & Hoeksema 1998), e.g. a photosynthesiser exchanging organic carbon for inorganic carbon with a heterotroph (Kuhl *et al*. 1996). And a recent simulation suggested that natural selection operating on communities of microbes can promote such positive interactions (Williams & Lenton 2008). It requires that associations are fairly stable, however, so that the benefits from investing in the other species feed back on the investing cell or its descendants (partner-fidelity feedback: Sachs *et al*. 2004, Foster & Wenseleers 2006).

Enforced

In many societies, including our own, conflict is reduced by coercion that forces individuals to comply (Wenseleers & Ratnieks 2006). We understand relatively little of mechanisms of coercion in microbes, but there are some interesting candidates. One example is conjugation. If a cooperative secretion is encoded on a plasmid, then plasmid transfer will force other cells to secrete (Smith 2001). There is also toxin secretion in the bacteria *B. subtilis* (Gonzalez-Pastor *et al*. 2003) and *M. xanthus* (Wireman & Dworkin 1977) that kills some cells and provides nutrients for spore formation. One must be careful here, however, as toxin secretion can obviously also signify raw conflict, and not its resolution. We need more data before we can tell if, in nature, the secretion of these toxins really promotes the total reproduction of the bacterial group (conflict resolution: Ratnieks *et al*. 2006) or simply allows one strain to monopolise the resources of another (plain old conflict).

The role of enforcement is perhaps clearest in social behaviours that occur between species. There are many examples from symbioses, where a microbe lives in or on a host species and the host is able to select the microbial cells that help it the most (partner

choice: Sachs *et al*. 2004, Foster & Wenseleers 2006). This includes fungus-growing ants that remove parasitic and foreign fungi (Currie & Stuart 2001, Mueller *et al*. 2004, Poulsen & Boomsma 2005) with the help of a streptomycete bacterium that secretes antifungals (Currie *et al*. 1999), and leguminous plants that direct resources to the roots with the most industrious nitrogen-fixing bacteria (Kiers *et al*. 2003, Simms *et al*. 2006). Even your intestine possesses mechanisms, some more subtle than others, which presumably tend to favour more cooperative bacteria and get rid of the less favourable ones. The subtle mechanisms include the still poorly understood effects of the immune system upon the microbial flora (Backhed *et al*. 2005, Ryu *et al*. 2008), and the less subtle mechanisms have been experienced by anyone who has travelled to a foreign country with a very different diet.

Successful coercion, however, requires that the recipient cannot easily escape its effects. Just as pleiotropy can internally constrain against cheating, it can also prevent escape from coercion. High-nutrient slime-mould cells (Fig. 13.6) induce low-nutrient cells to become stalk cells by secreting a chemical DIF-1, and avoiding DIF-1 is not an option: mutant cells that do not respond to DIF-1 also fail to enter the spore head to become spores (Foster *et al*. 2004). There are host–symbiont examples as well: the bobtail squid that hosts *V. fisheri* bacteria in its light organ creates an environment that enables fluorescent strains to outcompete non-fluorescent ones (Visick *et al*. 2000).

13.3.2 Indirect effects: kinship

Strain mixing

Personal benefits are an easy way to promote cooperation and make microbes behave as a unified group. However, just as important are benefits to related cells, or kin. Relatedness here means not genealogical relatedness, as in the statement 'rats and bats are related, they are both mammals', but rather relatedness among individuals within a species, as in 'I am related to my sister Gillian'. Simply put, if a group of microbes are recently derived from a single progenitor, their evolutionary interests are perfectly aligned, like the cells in your body. This means that selection can lead to dramatically altruistic traits, like cell death, when this allows a cell to pass on the genes that they carry in common with other individuals (indirect genetic benefits: Chapter 6). It does not matter, evolutionarily speaking, which cells in a clonal group get to reproduce, so long as the group performs well overall.

The key theoretical corollary is that anything that breaks the genetic correlation among group members will promote competition and conflict (Hamilton 1964). This holds, whether the group is the bounded aggregation of a slime mould or a more nebulous biofilm, so long as grouping is defined by the scale of social interactions (Grafen 2007). The importance of genetic relatedness among cells is supported by several studies that have mixed up microbes. These have shown that mixing strains promotes rapid wasteful growth in bacterial viruses (Kerr *et al*. 2006) and the success of cheater mutants, which has been seen in many contexts including yeast enzyme secretion (Greig & Travisano 2004), *P. aeruginosa* iron scavenging (Griffin *et al*. 2004), *P. aeruginosa* quorum sensing (Diggle *et al*. 2007b), and the development of *M. xanthus* (Velicer *et al*. 2000) and *D. discoideum*, where a myriad cheater mutants have now been found (Ennis *et al*. 2000, Santorelli *et al*. 2008). But why does a cheater do better at low relatedness? Consider the fate of a rare cheater mutant that has just arisen in a natural population of slime moulds (Fig. 13.6; Gilbert *et al*. 2007). If many strains of *D. discoideum* mix together (low relatedness), the cheater cells will mostly meet cooperator cells and do well. But when aggregates form from a single strain, as they often do, the cheaters will have no one to exploit and will do badly: cheating does not pay when you are surrounded by relatives. Similar principles apply to multi-species cooperation, only now one needs not only low mixing *within* species, but also *between* species, because this allows enough time for investments in a partner species to provide feedback benefits (Foster & Wenseleers 2006).

One does not have to wait for evolutionary time scales to see strain mixing affecting microbial groups. This is particularly true when cells can pinpoint

non-relatives, such as spitefully secreting a toxin to kill them (Gardner & West 2004, Gardner *et al*. 2004). With toxins, mixing immediately leads to problems: spore production is often decreased when *M. xanthus* strains are mixed (Fiegna & Velicer 2005) and mixing two bacteriocin-producing bacteria limits their ability to infect a host (Massey *et al*. 2004). The harmful effects of mixing can also be more subtle: slugs containing multiple strains of *D. discoideum* migrate poorly (Foster *et al*. 2002, Castillo *et al*. 2005).

If mixing is costly, then why do it? For some, the benefits of a large group will outweigh the costs, but others limit their mixing. A sister species of *D. discoideum*, *D. purpureum*, preferentially aggregates with cells of the same strain (Mehdiabadi *et al*. 2006), and strains of the bacterium *Proteus mirabilis* create an inhibition zone with other strains when swarming (Gibbs *et al*. 2008). In addition, so-called green-beard genes (Dawkins 1976), which code for cell-to-cell adhesion proteins, allow microbial cells that express the adhesion gene to preferentially associate with other expressing cells, while excluding non-expressers from aggregations. Such genes are likely to have promoted kinship in the early evolution of *D. discoideum* aggregation (Queller *et al*. 2003), and remain important today in *S. cerevisiae*, where only some strains express its key adhesion gene in natural populations (Smukalla *et al*. 2008). Quorum sensing can achieve similar effects. As we have seen, many beneficial secretions are only secreted when cells have grown to reach high density, which in biofilms may make a good proxy for being surrounded by relatives (Xavier & Foster 2007). And *B. subtilis* has taken this one step further by developing strain-specific quorum sensing (Tortosa *et al*. 2001, Ansaldi *et al*. 2002).

Mutation

One fundamental difference between microbes and more familiar social organisms such as insects, birds and mammals is probably the relative importance of mutation within groups (West *et al*. 2006). This is exemplified by the evolution of the bacterium *P. aeruginosa* in the cystic fibrosis lung (Smith *et al*. 2006). Over the years, strains arise that lose function in many genes important for normal social life, including motility, attachment and, as we have seen, quorum sensing (D'Argenio *et al*. 2007, Sandoz *et al*. 2007). But mutation-driven evolution in microbes does not need years. Bacteria sitting in a flask on a bench will evolve cooperation and lose it again to cheating in a matter of days. One of the more friendly pseudomonads, *Pseudomonas fluorescens*, has a solitary smooth strain that swims around in the broth but will rapidly mutate to generate a second wrinkly strain, so-called because it secretes sticky stuff that makes its colonies look, well, wrinkly (Fig. 13.1b shows a similar case from another species). The wrinklies cooperate with each other and form a mat that suffocates the smooth cells below them (Fig. 13.1c), only to have new mutant smooth cells appear in their midst that sink the whole lot (Paul B. Rainey's profile; Rainey & Rainey 2003, Brockhurst *et al*. 2007) in a turbid tragedy of the commons (Rankin *et al*. 2007b).

Global mutation rate is also important. If mutation towards cheating occurs more often than towards cooperation, high mutation rate will tend to promote conflict: *Pseudomonas aeruginosa* strains that mutate rapidly (Oliver *et al*. 2000) produce more cheaters (Harrison & Buckling 2005). A high mutation rate then can reduce genetic relatedness in a group and lead to the emergence of cheaters and conflict. Moreover, the starting levels of genetic relatedness in a microbial group can interact with this process and affect the evolution of mutation rates. Growing strains of *P. aeruginosa* in isolation (high relatedness) favours a low mutation rate, because strains are less likely to produce cheater mutants that harm the group (Harrison & Buckling 2007). By contrast, growing mixtures of strains (low relatedness) can, at least temporarily, favour a higher mutation rate, as now the rise of cheater mutants harms not only the source strain but also its competitor. A bacterial group with a low relatedness among cells is prone to processes that drive relatedness even lower.

Mutation in key genes then can simultaneously reduce relatedness and produce cheaters that exploit others, even though cells may be genetically identical at every other locus. This brings us to an important point: when using relatedness to make evolutionary predictions about cooperation, one must focus on the

locus or loci that cause the social action (Chapter 6). This is less of an issue in animal societies because sexual recombination means that, on average, relatedness is identical across the whole genome: any locus is a proxy for the loci that really matter. But in a mutating asexual microbe, the relatedness among cells can differ sharply at different loci, which suggests that the loci may also differ systematically in their evolutionary interests, creating within-genome conflicts (Burt & Trivers 2006, Helantera & Bargum 2007).

A candidate worthy of a little speculation in this regard is the reliable production of multiple growth phenotypes by *P. aeruginosa* within biofilms (Fig. 13.4; Boles *et al*. 2004). This appears to be genetically determined, and diversification makes biofilms tougher, suggesting that the majority of the loci in the genome, which do not alter, will benefit. However, if there are loci that must mutate to cause diversification, there is the potential for conflict among the resulting alleles, and also between these alleles and the rest of the genome. Examples such as this suggest that microbes may simultaneously indulge in the benefits of high relatedness at some loci (cooperation), while at the same time benefiting from variability at others (social heterosis: Nonacs & Kapheim 2007).

13.4 Conclusions and future directions

Recent years have seen the development of a host of so-called 'culture-independent' technologies for assessing microbial diversity. These detect a species directly from its RNA or DNA and remove the requirement that – in order to be detected – a species must grow in the laboratory setting. The new technologies have led to the realisation that many species were missed by traditional techniques and that natural microbial communities frequently contain hundreds of species living in close proximity. In many ways, therefore, the study of microbial behaviour and social interaction are only now beginning.

The newly recognised species diversity in microbial communities hints at an equally broad range of behavioural diversity that remains to be uncovered. There is therefore considerable scope for taking both known and unknown species and simply documenting their social behaviours. Here I highlight three key questions that can be asked of a new microbial group, with the important caveat that any detailed study will always require that one can first get cells to grow in the laboratory setting. These questions are borrowed from a recent review on biofilms (Nadell *et al*. 2009).

Function: what are the constituent genes and phenotypes that drive microbial social traits? Identifying the genes that underlie social traits allows one to dissect complex group traits – like biofilms – into their constituent behaviours. This enables the systematic study of the costs and benefits of a given behaviour (below) and an assessment of the evolutionary forces acting upon traits by comparing DNA sequence variation within and between species (Smith *et al*. 2005).

Cooperation: what are the costs and benefits of microbial social behaviours? In order to understand the evolution of microbial behaviours, we need to identify which cells are affected by each behaviour. In particular, following the logic of Chapter 6, the two key questions are (1) what are the costs and benefits of a behaviour to the cell that expresses it, and (2) what are the costs and benefits of a behaviour to cells other than the individual that expresses it? A key experiment in this regard is to mix mutants that do not express a social trait with cells that do, in order to evaluate the potential for cheating (e.g. Gilbert *et al*. 2007). Only by asking such questions of a whole range of microbial behaviours will we get an idea of how many involve true altruism, whereby one cell helps another at a fitness cost to itself (Table 13.1).

Ecology: which strains and species are in natural microbial groups, and how are they arranged? We need a much better knowledge of microbial ecology in order to understand how costs and benefits measured in the laboratory translate into real fitness effects in the wild. An important start, which is well under way, is to identify the species and strains within natural communities. But to answer questions of social evolution one

must go further still and assess the microscopic distribution of the different genotypes. While challenging, such fine-scale assessments in natural groups will provide estimates of genetic relatedness and, importantly, the spatial scale at which one can expect cells to act as a unified group of common interest (Fig. 13.2).

As you next stroll through a sun-dappled woodland, consider the humble microorganism. For they thrive all around us, be it on the surface of a water droplet, in the depths of the soil, or nestled inside the tiny pores of a leaf. Wherever they live, they are often packed together with many other species and strains. Here social interaction is rife: cells jostle for position, grab, secrete, poison and even shape-shift as the need arises. Only now is the true extent of the microbe's social sophistication becoming clear, and with it comes a rich opportunity to unravel the evolutionary paths that brought them there. But she who studies their societies faces a bewildering complexity, not only in terms of the diversity of those that interact, but also in the potential for rapid evolutionary responses when they do. There is some solace, however, in the knowledge that familiar principles of sociobiology are emerging from these microbial melees. Kinship, coercion and constraints are all important, and all-important. And in the end we know that each cell has its own interests at heart, and will act to safeguard the reproductive interest of itself and its clone-mates. There can be no doubt that microbial communities are shaped by the individual struggles that face all organisms, but there is room for some romanticism too. For with any struggle comes the benefit of alliances, familial or otherwise, that invest in a shared common good. Some microbes will even die for the cause, and rupturing to secrete a toxin can be simultaneously altruistic to those that are immune and spiteful to those that are not. In applying terms like altruism to microbes, however, we are met with something of a paradox. As Spencer realised long ago, the individual microorganism seems destined to be both selfish *and* altruistic because, in its eagerness to divide, it faces the ultimate metaphysical sacrifice: a loss of self.

> The simplest beings habitually multiply by spontaneous fission. Physical altruism of the lowest kind, differentiating from physical egoism, may in this case be considered as not yet independent of it. For since the two halves which before fission constituted the individual, do not on dividing disappear, we must say that though the individuality of the parent infusorium or other protozoon is lost in ceasing to be single, yet the old individual continues to exist in each of the new individuals.
>
> Herbert Spencer, *The Data of Ethics* (1879)

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