Social behaviour in microorganisms

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Summary

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. Indeed like most species, almost everything that a microorganism does has social consequences; simply dividing can consume another’s resources. Microbes also secrete a plethora 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 like any society, microbial conflicts are mediated by three key processes: constraints on rebellion, coercion that enforces compliance, and kinship whereby cells direct altruistic aid toward clone-mates.
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 popularizing 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). 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 (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 lay 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 1) (biofilms, Kolter and Greenberg 2006). 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 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 and 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 1). “Social behaviour” simply means a behaviour that affects another cell’s evolutionary fitness (Table 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).

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 (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 (West et al. 2006) (Table 1). 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 due 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.

This all 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 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 neighbour 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", Rankin et al. 2008). But returning to microbes; many have the ability to switch from aerobic respiration to fermentation that produces energy more rapidly when oxygen is limited. However, fermentation is also inefficient and yields much less energy overall (Pfeiffer et al. 2001; Pfeiffer and Schuster 2005; Novak et al. 2006). Competing cells therefore are expected to more often make use of fermentation (MacLean and Gudelj 2006) while cooperative systems, including multicellular organisms, are more likely to be altruistically aerobic, whereby each cell slows its growth now to allow other cells to have food in the future (Kreft 2004; Kreft and 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 and Kolter 2001) (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 evolutionary fitness (Fig 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 and 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 and Traviseo 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 carrier” in Greek) (Fig 2). These dissolve insoluble iron and allow the bacterium to combat a common vertebrate response known as localized 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 and Travisano 2004; Smith et al. 2005). This may indicate shifting ecological demands (Brockhurst et al. 2007; Foster and Xavier 2007), or even an evolutionary arms-race in which cooperators attempt to evolve products that cannot be used by cheaters (Tuemmler and Cornelis 2005). But it is not all darkness and disease. Many microbes provide metabolic assistance by producing extracellular compounds or resources that their host organisms cannot make. This includes our own gut fauna, and also antifungal bacteria that protect leafcutter 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 and 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 and 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 a 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 (Noda et al. 2003) (Fig 3). 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; Konig et al. 2007). Meanwhile, some spirochetes rely on cross-feeding from yet other bacteria in the termite gut (Graber and Breznak 2004)…

Shared products can be structural too. 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 and 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 are complex mixtures that probably protect the cells from various stresses (Crespi 2001; Velicer 2003; Hall-Stoodley et al. 2004; Foster 2005; Keller and 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 4) (Foster and Xavier 2007; Xavier and 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 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. Only then, this second strain can be replaced by the original susceptible cell type that produce 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 may represent a general principle for the maintenance of biodiversity (Durrett and 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 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 cell 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 1B) that actively secretes a toxin that kills non-expressing cells in the surroundings (Gonzalez-Pastor et al. 2003; Dubnau and Losick 2006), and viral-induced cell death in *P. aeruginosa* biofilms (Allesen-Holm et al. 2006) (Fig 2B), which have both been interpreted as purely cooperative behaviours.

13.2.3 Communication

Microbes also use secretions to talk to 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 and 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 synchronize their intracellular physiologies, even though cell division is not synchronized (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 in both bacteria (Miller and Bassler 2001; Keller and Surette 2006; Diggle et al. 2007a) and fungi (Hogan 2006), and involves a wide variety of secreted compounds known as autoinducers, including some packaged in vesicles (Mashburn and Whiteley 2005). All exploit the same simple bit of physics: 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 characterized 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 positive-feedback loop whereby it induces an increase in its own production, hence the term autoinducer. Since then, the name has spread to quorum-sensing molecules that do not auto-induce, 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 and 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, the same way that cells can cheat on a shared enzyme (Fig 2a) (Brown and 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 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 and 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 and Bassler 2003). How often this represents active signalling on the part of secreting cells is not known (Keller and Surette 2006; Diggle et al. 2007a) but some species, including \textit{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 and Surette 2006): \textit{E. coli} actively takes up AI-2, and thereby prevents \textit{Vibrio cholerae} from secreting proteases that it uses during cholera gut infection (Xavier and Bassler 2005). Meanwhile, the dental bacterium \textit{Veillonella atypica} secretes a compound that induces \textit{Streptococcus gordonii} to release lactic acid, which \textit{V. atypica} then feeds on (Egland et al. 2004).

\section*{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 and Nielsen 2005; Narra and 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.

\textit{V. cholerae} becomes naturally competent when growing in a biofilm on arthropod exoskeletons (Meibom et al. 2005) and competence in \textit{B. subtilis} (Fig 1B) involves both quorum-sensing (Magnuson et al. 1994) and differentiation (below). Perhaps the most interesting case though comes from \textit{Streptococcus} species, who 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 then to incorporate its DNA into your own and partially become it? The answer may lie in the recognizing 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 Roman’s adopting Greek gods.

Transport of DNA between cells is even more directed when it is driven by plasmids (conjugation) and viruses (transduction). Plasmids are the relatively-small
circular pieces of DNA carried by bacteria. Despite their small size, they 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 and 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 and Townsend 2005; Thomas and 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 1) in which cells secrete all manner of products, including the slimy polymers that form a structural matrix around the cells (Fig 4) (Branda et al. 2005; Parsek and Greenberg 2005). 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 no longer need it (Hammer and 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 and Bassler 2005). The best studied multi-species 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 another for attachment and growth (Palmer et al. 2001).

Living in a dense biofilm presents challenges, including intense nutrient competition that threatens to slow growth (Stewart 2003; Xavier and Foster 2007) (Fig 4A/B). So why make one? The answer is likely to vary between species (Hall-
Stoodley and 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 (Rainey and Rainey 2003; Brockhurst et al. 2007) (Fig 1C), 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 and Greenberg 2005; Keller and Surette 2006), and they are tough: biofilms are the scourge of medicine and industry where they clog, contaminate, and cake both 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 that flocculation in the yeast \textit{S. cerevisiae}, whereby cells aggregate together at high densities, provides protection against stresses and antifungal agents (Smukalla et al. 2008).

Bacterial biofilms can also be dynamic environments as strains struggle to get the best nutrients, both by altruistically pushing descendent cells with slime (above, Fig 4C) (Xavier and Foster 2007) and by active cell movement (Klausen et al. 2003) (Fig 4D). 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 \textit{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 \textit{Myxococcus xanthus} that had been mutated to stop pili-based swarming was able to re-evolve motility using a different mechanism (Velicer and Yu 2003) (Fig 5A). 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 uniform, such as differentiation into swarming cells in \textit{Salmonella enterica}, which become elongated and carry many flagella for efficient propulsion (Fig 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 (Xu et al. 1998) (Fig 4A/B). 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 and 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 (Gomer and Firtel 1987) (Fig 6). 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 like yeast is random acts of gene silencing through DNA methylation or chromatin modification (Rando and 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 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 byproduct 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 functional diversification. One idea is that cells are hedging their bets on the future (Kussell and Leibler 2005). That is, if there may or may not be a 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 cell in your body and 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 and 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 and 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 6A). The slug sheds cells as it goes that colonize nearby patches (Kuzdzal-Fick et al. 2007) (Fig 6B) 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 6C). Analogously, cells of the bacteria *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 and 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 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 maximize 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 and 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, as such, mechanisms that limit conflicts must 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 and 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 6B), but not if they then force it to make 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 and Velicer 2004). If cooperative systems that avoid cheating persist longer than those that do not, over time there will be an enrichment for systems with pre-existing constraints on the origin of cheaters (Foster et al. 2007; Rankin et al. 2007). There are several mechanisms that might cause these constraints including reduced mutation rate (below, Harrison and 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 and 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 and Roze 2001). In this vein, natural selection for cooperation has been shown to lead to reduced global mutation rate in P. aeruginosa (below, Harrison and Buckling 2007). Constraints on cheating can also arise through an arms-race like process in which a cheater strain paves the way for 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. 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 (Fiegna et al. 2006); 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 specialize on a different resource than the competing species. This can have two knock-on effects. Firstly, 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 and Hoeksema 1998), e.g. a photosynthesizer 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 and Lenton 2008). It requires that associations are fairly stable, however, so that the benefits from investing in the other species feedback on the investing cell or its descendents (partner-fidelity feedback, Sachs et al. 2004; Foster and Wenseleers 2006).

**Enforced**

In many societies, including our own, conflict is reduced by coercion that forces individuals to comply (Wenseleers and 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 and Dworkin 1977) that kills some cells and provide 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 monopolize 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, where the host is able to select the microbial cells that help it the most (partner choice, Sachs et al. 2004; Foster and Wenseleers 2006). This includes fungus-growing ants that remove parasitic and foreign fungi (Currie and Stuart 2001; Mueller et al. 2004; Poulsen and 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, by which it can favour more cooperative bacteria and rid itself of the less favourable ones (Backhed et al. 2005; Ryu et al. 2008). Successful
coercion though requires that the recipient cannot easily escape its effects. Just as pleiotropy can internally constrain against cheating, it can prevent escape from coercion. High-nutrient slime mould cells (Fig 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 in yeast enzyme secretion (Greig and Travissano 2004), P. aeruginosa iron-scavenging (Griffin et al. 2004), P. aeruginosa quorum-sensing (Diggle et al. 2007b), and in the development of M. xanthus (Velicer et al. 2000) and D. discoideum where a myriad of 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 (Gilbert et al. 2007) (Fig 6). 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 do badly: cheating does not pay when you are surrounded by relatives. Similar principles applies 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 and 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 and 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 and 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 (Mehdiabadi et al. 2006), preferentially aggregates with cells of the same strain, 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 because 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 and 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 like 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 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 1C), only to have new mutant smooth cells appear in their midst that sink the whole lot (Rainey and Rainey 2003; Brockhurst et al. 2007) in a turbid tragedy of the commons (Rankin et al. 2008).
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 and 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 and 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 and Trivers 2006; Helantera and 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 (Boles et al. 2004) (Fig 4). 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 like 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 and Kapheim 2006).

### 13.4 The secret social lives of microorganisms

As you next stroll through a sun-dappled woodland, consider the humble microorganism. For their tiny number 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; both 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 though 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. Yes, 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, 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, though, we are met with something of a paradox. As Spencer realized long ago, the individual microorganism seems destined to be both selfish \textit{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.” Spencer (1879), The Data of Ethics

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13.6 References


Box 1: Some key players in microbial social evolution

**Dictyostelium discoideum**: this slime mould or social amoebae 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 6).

**Bacillus subtilis**: a spore-forming soil bacterium that forms tough biofilms on agar and on the surface of growth medium (Fig 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 (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 sometime associated with social motility, an example of group predation (Fig 5A).

**Pseudomonas**: *P. aeruginosa* is a pathogenic bacterium that is sometimes found in soil and often in the clinic (Fig 2B). While best known for its ability to cause complications in the lungs of cystic fibrosis patients, it can cause problems in any immunosuppressed patient. *P. aeruginosa* also forms robust biofilms (Fig 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 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 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 colonization 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, *Vibrio fisheri*, lives mutualistically in the light organ of the bobtail squid and produces light under quorum-sensing regulation.
Table 1. Definitions of social behaviour based upon the effects on personal lifetime reproduction (direct fitness). See also Chapter 6.

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Cooperation  Competition/ Conflict
Figure 1. Microbial groups. A) Large microbial biofilm in a Massachusetts river, US. 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 blue area shows dead cells on the outside but these protect the cells on the inside. Figure A by the author, B by Hera Vlamakis, C by Andrew Spiers, D by Kevin Verstrepen.
Figure 2. Secreted products in microbes. A) Cooperative secreted products and cheating: the grey cells secrete a product that benefits all cells but the red cells do not and save energy in the process. The red cells cheat and outgrow the grey cells. B) Different strains of the bacterium *P. aeruginosa* growing on agar displaying different pigmentation that result, at least in part, from differences in pyoverdin (green) and pyocyanin (blue) secretion that both function in iron scavenging. The far right strain also shows the characteristic spotting of viral driven cell-lysis. Photos by the author.
Figure 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 (Konig et al. 2007). Photo A by Judith Korb, Photo B by Helmut König.
Figure 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 (green cells are growing quickly and blue ones slowly). B) A biofilm of *P. aeruginosa* in which metabolically active cells have been stained yellow C) The effect of polymer secretion on success within a biofilm. The simulation shows red cells that secrete the yellow polymer and altruistically push their descendents up into the oxygen, which suffocates the blue cells that do not secrete (Xavier and Foster 2007). D) The effect of motility on success within a biofilm. Confocal image of a *P. aeruginosa* biofilm with a yellow motile strain that moves towards the top of the biofilm and a non-motile blue strain that does not (Klausen et al. 2003). Images A and C from Joao Xavier, B from Phil Stewart and D from Tim Tolker-Nielsen.
Figure 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, that 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 dead stalk 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 wildtype cells the mutant is competitively superior and produces more spores (Ennis et al. 2000; Gilbert et al. 2007). Photos by the author.