CHAPTER 2

Communication in bacteria

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2.1 Introduction: communication in a unicellular world

In 1905, the pioneering plant pathologist E. F. Smith suggested that 'a multiple of bacteria are stronger than a few and thus by union are able to overcome obstacles too great for the few' (Smith 1905). This was for the time a remarkable statement, because until recently it was considered by most microbiologists that bacterial cells were unicellular organisms that existed in isolation from each other. It is now well established that bacteria are highly interactive and possess an extraordinary repertoire for intercellular communication and social behaviours such as group migration, conjugal plasmid transfer (sexual transfer of genetic material between cells), antibiotic resistance, biofilm maturation (development of 'slime cities'), and virulence which, although not a social trait, can be a consequence of social behaviour (Williams *et al.* 2007).

Indeed, some workers have suggested that these behaviours are similar to those observed in social insects, vertebrates, and humans. For example, *Myxococcus xanthus* cells exhibit socially dependent swarming across surfaces (Velicer and Yu 2003) which allows the population to seek out bacterial prey in a manner reminiscent of hunting packs of wolves (Dworkin 1996; Crespi 2001). In a similar fashion, biofilms (a collection of bacterial cells enclosed in a polysaccharide matrix) have been likened to ant nests and beehives (Crespi 2001; Diggle *et al.* 2007b). Furthermore, bacteria such as *Pseudomonas aeruginosa* can modulate the immune response, reminiscent of helminth parasites, and antibiotic resistance due to the production of extracellular enzymes (e.g. β-lactamase) could be considered to be a group defence mechanism (Diggle *et al.* 2007b).

Perhaps the paradigm for bacterial cooperation and communication can be seen in the diverse **quorum sensing** (QS) systems found in both Gram-negative and Gram-positive bacteria (Diggle *et al.* 2007a; Williams *et al.* 2007). QS describes the phenomenon whereby the accumulation of 'signalling' molecules in the surrounding environment enables a single cell to sense the number of bacteria (cell density), and therefore the population as a whole can make a coordinated response. The signal produced regulates its own production (autoinduction) and so this leads to a positive-feedback response and greatly increased signal production. At critical cell densities, the binding of a regulator protein to the signal leads to the switch on of genes controlled by QS and a coordinated population response.

It is important to note that many studies on QS in bacteria have been performed under laboratory conditions, and it needs to be determined whether QS is an artefact of laboratory growth (Redfield 2002; Hense *et al.* 2007). It is possible that this may be the case for certain organisms, but it has been shown, for example, that *P. aeruginosa* makes QS signal molecules in the lungs of cystic fibrosis patients (Collier *et al.* 2002; Middleton *et al.* 2002). Despite this, it is still not known whether QS is important in the development and establishment of chronic infections in this population. Therefore, the idea that QS is for the 'common good' of the bacterial population has yet to be significantly

Table 2.1 Bacterial cooperative behaviours known to be regulated by QS systems

QS-controlled behaviour	Bacterial species
Biofilms	*Aeromonas hydrophila, Burkholderia cenocepacia, Pseudomonas aeruginosa, Pseudomonas putida, Serratia liquefaciens*
Exoproteases	*Aeromonas hydrophila, Aeromonas salmonicida, Burkholderia pseudomallei, Pseudomonas aureofaciens, Serratia liquefaciens*
Plasmid conjugation	*Agrobacterium tumefaciens, Rhizobium leguminosarum*
Exoenzymes	*Burkholderia cenocepacia, Erwinia carotovora, Chromobacterium violaceum, Pseudomonas aeruginosa, Serratia* spp. ATCC 39006, *Serratia proteamaculans*
Swarming motility	*Burkholderia cenocepacia, Pseudomonas aeruginosa, Serratia liquefaciens, Yersina enterocolitica, Yersinia pseudotuberculosis*
Siderophore production	*Burkholderia cenocepacia*
Virulence	*Agrobacterium vitiae, Burkholderia cenocepacia, Burkholderia pseudomallei, Burkholderia mallei, Erwinia carotovora, Pseudomonas syringae, Pseudomonas aeruginosa*
Pigment production	*Chromobacterium violaceum, Pseudomonas aureofaciens, Pseudomonas chlororaphis, Serratia* spp. ATCC 39006, *Serratia marcescens*
Antibiotics	*Erwinia carotovora, Serratia* spp. ATCC 39006
Exopolysaccharides	*Pantoea stewartii, Pseudomonas syringae*
Aggregation	*Rhodobacter sphaeroides, Yersinia pseudotuberculosis*
Swimming motility	*Yersinia enterocolitica, Pseudomonas syringae*
Root nodulation/symbiosis	*Rhizobium leguminosarum, Sinorhizobium meliloti*
Biosurfactant production	*Pseudomonas aeruginosa, Serratia liquefaciens, Serratia marcescens*
Sliding motility	*Serratia marcescens*
Bioluminescence	*Vibrio fischeri*

proven. That aside, many of the behaviours regulated by QS appear to be cooperative and could be described as public goods, for example exoenzymes, biosurfactants, antibiotics, and exopolysaccharides (Table 2.1).

The importance of QS to a bacterium can be seen when studying the opportunistic pathogen *P. aeruginosa*. In this organism, a hierarchical QS system has been estimated to regulate at least 6% of the genome (Hentzer *et al.* 2003; Schuster *et al.* 2003; Wagner *et al.* 2003) which is a possible reason why *P. aeruginosa* is so highly adaptable and able to inhabit a wide range of diverse environmental niches.

It is often assumed in the microbiology literature that QS behaviour is cooperative and is for the good of the population as whole (Shapiro 1998; Henke and Bassler 2004) and little attention has been given to the evolutionary implications of QS. Understanding cooperative behaviour is one of the greatest challenges faced by evolutionary biologists, and the dictum of the survival of the fittest makes it unclear why one organism should behave for the good of another (Hamilton 1964). This chapter will review QS in bacteria and integrate this with the literature on animal signalling. We will discuss the nature of QS signals and signalling between single species and mixed species (bacterial cross-talk) and whether QS is truly cooperative. We will also explore whether QS in bacteria can be used to answer fundamental questions, such as how social behaviours can be maintained in natural populations.

2.2 When is a signal not a signal?

As will be described later, many diverse compounds have been identified as bacterial cell-to-cell QS signal molecules. Furthermore, interactions between different species of bacteria, and even between prokaryotes and eukaryotes, have also been widely described. There are several characteristics that a typical QS signal should display: (1) the production of the QS signal takes place during specific stages

of growth or in response to particular environmental changes; (2) the QS signal accumulates in the extracellular environment and is recognized by a specific bacterial receptor; (3) the accumulation of a critical threshold concentration of the QS signal generates a concerted response; and (4) the cellular response extends beyond the physiological changes required to metabolize or detoxify the molecule (Winzer *et al.* 2002). Even taking these factors into consideration, it is also important to define what a **signal** is using terminology that is accepted amongst evolutionary biologists when discussing signalling between higher organisms (Keller and Surette 2006; Diggle *et al.* 2007b) (see also Chapter 1).

In a seemingly simple scenario, when we see cell A produce a substance X that elicits a response in cell B it is tempting to conclude that the substance produced is a signal, i.e. cell A is trying to tell cell B something. The word 'signal' is widely used to define such substances in the context of QS, or communication between bacterial cells. However, broad use of this term can be misleading and obscure the details of the interaction between cells that it attempts to describe. This has been well illustrated by research on communication and signalling in animals, where considerable confusion has arisen through different researchers using the same term to mean different things, or different terms to mean the same things (Maynard Smith and Harper 2003).

Confusion over terminology can be avoided if the different kinds of interactions that we observe when cell A elicits a response in cell B are differentiated, depending upon their consequences for cell A and cell B (Table 2.2) (Maynard Smith and Harper 2003). Specifically, a signal is defined as 'characters that evolve in a signaller in order to provide information to a receiver, aiming to change the behaviour of the receiver to the benefit of the signaller' (see Chapter 1). This definition distinguishes a signal from a **cue**, where the production of substance X by cell A has not evolved *because* of its effect on cell B. For example, substance X may be a waste product produced by cell A that is detected by cell B. To demonstrate that substance X is a signal and not a cue it is necessary to show that it evolved because of the response it elicits. If the production of substance X by cell A forces a costly response from cell B we differentiate this from signalling and term it coercion or chemical manipulation.

Table 2.2 Different types of communication identified by their fitness consequences on the sender and receiver

	Evolved because of effect on sender (Cell A)	Benefits receiver to respond (Cell B)
Signal	+	+
Cue	–	+
Coercion	+	–

Beneficial (+), Costly (–).

Do these semantic points really matter? The answer is yes, for two reasons. Firstly, it is important for general understanding if there is a consensus on the use of terms. This is a lesson hard-learned by biologists working on signalling in higher organisms (Maynard Smith and Harper 2003), as well as more generally in the field of social evolution (West *et al.* 2007b). Secondly, and more importantly, we can make very different predictions about the behaviour of bacterial cells depending on whether they are communicating by a signal, a cue, or coercion (Table 2.2). For example, if a molecule is a signal, then we can say several things:

1. It is beneficial to cell B to respond.
2. The response of cell B benefits cell A.
3. It might be possible for a signaller to cheat in the amount of signal that it produces either to: (a) free-ride on the back of other signallers (avoiding the cost of producing substance X, i.e. signal negative), (b) manipulate responders (signal can become coercive), or (c) not respond to the presence of signal and therefore not produce signal-controlled public goods (signal blind).
4. There must be some mechanism that provides a shared interest to cells A and B, otherwise cheats would invade and make the signalling unstable—later we discuss how **kin selection** provides a solution to this problem.
5. A signalling system is likely to be more complex than a system involving a cue, to remain stable in the face of evolution for individuals to make less substance X or for individuals to respond less.

2.3 The discovery of cell-to-cell communication in bacteria

Whilst the term 'quorum sensing' has only been in use since 1994 (Fuqua *et al.* 1994), cell-to-cell communication in bacteria has an experimental history that dates back to the early 1960s. Early work on fruiting body formation in *M. xanthus* (Mcvittie *et al.* 1962) and on streptomycin production in *Streptomyces griseus* (Khokolov *et al.* 1967) challenged the common view that bacteria behaved as isolated single cells.

One of the earliest reports of a classical cell density-dependent phenotype was by Nealson *et al.* (1970) who showed that the addition of spent culture supernatants of the marine luminescent bacterium *Vibrio fischeri* (formally *Photobacterium fischeri*) to low-density cultures of the same organism induced the production of bioluminescence due to the presence of a substance they termed an autoinducer (Nealson *et al.* 1970). When in a confined area such as a flask, or in symbiosis in a light organ found in certain species of squid, the autoinducer molecules accumulate to a critical concentration (usually at high bacterial cell densities) which, in turn, induces expression of the genes responsible for bioluminescence.

The autoinducer responsible for the regulation of bioluminescence was later identified as *N*-(3-oxohexanoyl) homoserine lactone (3-oxo-C6-HSL) (Eberhard *et al.* 1981). The structural and regulatory genes necessary for bioluminescence and 3-oxo-C6-HSL production were identified and termed the *lux* regulon (Engebrecht *et al.* 1983). This regulon is organized into two divergently transcribed operons (operons are units of coordinated gene activity which regulate protein synthesis in prokaryotes). The leftward operon comprises the *luxR* gene which encodes the transcriptional regulator protein LuxR. The rightward operon consists of six genes arranged as *luxICDABE*. The *luxI* gene encodes an autoinducer synthase responsible for the synthesis of 3-oxo-C6-HSL. The *luxCDABE* genes are involved in generating the products required for the luciferase reaction and the induction of bioluminescence. The genetic regulation of bioluminescence in *V. fischeri* is illustrated in Fig. 2.1. This elegant mechanism of gene regulation was thought to be a phenomenon restricted to bioluminescence production in a few marine *Vibrio* species; however, it is now known that this type of system is widespread in Gram-negative bacteria.

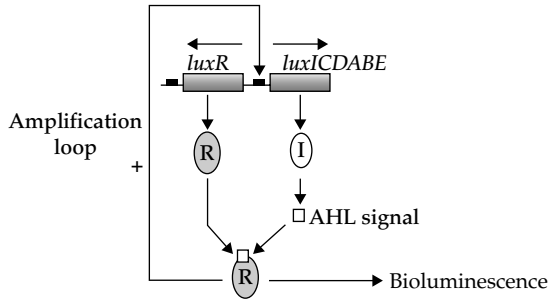

Figure 2.1 The LuxR /AHL-driven quorum sensing module of *V. fischeri*. LuxR is the AHL receptor and LuxI is the AHL signal synthase. Many bacteria possess multiple LuxR /LuxI /AHL modules which work in a similar manner.

In the early 1990s it was discovered that the production of the β-lactam antibiotic, 1-carbapen-2-em-3-carboxylic acid (carbapenem) by the terrestrial plant pathogen *Erwinia carotovora* was also regulated by 3-oxo-C6-HSL (Bainton *et al.* 1992a,b). This finding led to the intriguing possibility that many bacteria may use *N*-acylhomoserine lactones (AHLs) in order to regulate specific phenotypes. This was confirmed when Bainton *et al.* (1992a) used plasmid-based AHL-biosensors to detect AHL molecules from spent culture supernatants from *P. aeruginosa*, *Serratia marcescens*, *Erwinia herbicola*, *Citrobacter freundii*, *Enterobacter agglomerans*, and *Proteus mirabilis* (Bainton *et al.* 1992a). Since this work, many other Gram-negative bacteria have been shown to produce different types of AHL molecules and all have homologues of LuxI and LuxR proteins of *V. fischeri* (Table 2.3). AHL-mediated QS is responsible for the regulation of a wide variety of different phenotypes in these organisms.

Although the distribution of Gram-negative bacteria that produce AHLs is widespread, there are some Gram-negative species that have failed to exhibit any activity in any of the AHL biosensor assays available, for example *Escherichia coli* and *Salmonella* species. However, this does not mean that they are incapable of producing and sensing a signal, and Gram-negative bacteria often utilize alternative QS signal molecules. The

Table 2.3 LuxR/AHL-dependent QS systems in Gram-negative bacteria

Organism	Major AHL(s)	LuxR	LuxI	Phenotypes
Aeromonas hydrophila	C4-HSL, C6-HSL	AhyR	AhyI	Biofilms, exoproteases
Aeromonas salmonicida	C4-HSL, C6-HSL	AsaR	AsaI	Exoprotease
Agrobacterium tumefaciens	3-oxo-C8-HSL	TraR	TraI	Plasmid conjugation
Agrobacterium vitiae	C14:1-HSL, 3-oxo-C16:1-HSL	AvsR	AvsI	Virulence
Burkholderia cenocepacia	C6-HSL, C8-HSL	CepR, CciR	CepI, CciI	Exoenzymes, biofilm formation, swarming motility, siderophore, virulence
Burkholderia pseudomallei	C8-HSL, C10-HSL, 3-hydroxy-C8-HSL, 3-hydroxy-C10-HSL, 3-hydroxy-C14-HSL	PmlIR1, BpmR2, BpmR3	PmlI1, PmlI2, PmlI3	Virulence, exoprotease
Burkholderia mallei	C8-HSL, C10-HSL	BmaR1, BmaR3, BmaR4, BmaR5	BmaI1, BmaI3	Virulence
Chromobacterium violaceum	C6-HSL	CviR	CviI	Exoenzymes, cyanide, pigment
Erwinia carotovora subsp. carotovora	3-oxo-C6-HSL	ExpR, CarR	CarI (ExpI)	Carbapenem, exoenzymes, virulence
Pantoea (Erwinia) stewartii	3-oxo-C6-HSL	EsaR	EsaI	Exopolysaccharide
Pseudomonas aeruginosa	C4-HSL; C6-HSL, 3-oxo-C12-HSL	LasR, RhlR, QscR, VqsR	LasI, RhlI	Exoenzymes, exotoxins, protein secretion, biofilms, swarming motility, secondary metabolites, 4-quinolone signalling, virulence
Pseudomonas aureofaciens	C6-HSL	PhzR, CsaR	PhzI, CsaI	Phenazines, protease, colony morphology, aggregation, root colonization
Pseudomonas chlororaphis	C6-HSL	PhzR	PhzI	Phenazine-1-carboxamide
Pseudomonas putida	3-oxo-C10-HSL, 3-oxo-C12-HSL	PpuR	PpuI	Biofilm formation
Pseudomonas syringae	3-oxo-C6-HSL	AhlR	AhlI	Exopolysaccharide, swimming motility, virulence
Rhizobium leguminosarum bv. *viciae*	C14:1-HSL, C6-HSL, C7-HSL, C8-HSL, 3-oxo-C8-HSL, 3-hydroxy-C8-HSL	CinR, RhiR, RaiR, TraR, BisR, TriR	CinI, RhiI, RaiI	Root nodulation/symbiosis, plasmid transfer, growth inhibition; stationary phase adaptation
Rhodobacter sphaeroides	7-cis-C14-HSL	CerR	CerI	Aggregation
Serratia spp. ATCC 39006	C4-HSL, C6-HSL	SmaR	SmaI	Antibiotic, pigment, exoenzymes
Serratia liquefaciens MG1	C4-HSL, C6-HSL	SwrR	SwrI	Swarming motility, exoprotease, biofilm development, biosurfactant
Serratia marcescens SS-1	C6-HSL, 3-oxo-C6-HSL, C7-HSL, C8-HSL	SpnR	SpnI	Sliding motility, biosurfactant, pigment, nuclease, transposition frequency
Serratia proteamaculans B5a	3-oxo-C6-HSL	SprR	SprI	Exoenzymes
Sinorhizobium meliloti	C8-HSL, C12-HSL, 3-oxo-C14-HSL, 3-oxo-C16:1-HSL, C16:1-HSL, C18-HSL	SinR, ExpR, TraR	SinI	Nodulation efficiency, symbiosis, exopolysaccharide
Vibrio fischeri	3-oxo-C6-HSL	LuxR	LuxI	Bioluminescence
Yersinia enterocolitica	C6-HSL, 3-oxo-C6-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, 3-oxo-C14-HSL	YenR, YenR2	YenI	Swimming and swarming motility
Yersinia pseudotuberculosis	C6-HSL, 3-oxo-C6-HSL, C8-HSL	YpsR, YtbR	YpsI, YtbI	Motility, Aggregation

cabbage pathogen *Xanthomonas campestris* employs a low-molecular-weight diffusible factor unrelated to AHLs to regulate expression of virulence determinants such as extracellular enzymes and exopolysaccharide (Barber *et al.* 1997). Furthermore, another plant pathogen, *Ralstonia solanacearum*, uses a 3-hydroxypalmitic acid methyl ester as a volatile signal molecule (Clough *et al.* 1997). *Myxococcus xanthus* also produces non-AHL signals. This Gram-negative bacterium is capable of forming complex multicellular structures that play a role in starvation survival. In order to coordinate this, *M. xanthus* produces two different signals, the A-signal and the C-signal. The A-signal, produced under nutrient limitation and at high cell densities, is the first signal that triggers multicellular behaviour. Analysis has revealed that the A-signal is a mixture of amino acids and small peptides (Kuspa *et al.* 1992). Following the formation of a layer of cells triggered by the A-signal, the production of the C-signal gives rise to the next stages in the development process, cell aggregation and sporulation.

The molecules identified and the processes controlled in *M. xanthus* are very different from those associated with AHLs and there have now been multiple signalling systems described, using different chemical signals, in the same organism. For example, *P. aeruginosa* has been shown to produce two AHL-distinct classes of molecules (2-alkyl-4-quinolones and cyclic dipeptides) with signalling activity in addition to AHLs (Holden *et al.* 1999; Pesci *et al.* 1999; Diggle *et al.* 2006a). This suggests that the signal may be tailored to particular physiological or environmental conditions depending upon its physical properties. Some examples of bacterial QS signals can be seen in Fig. 2.2.

Signalling is not restricted to Gram-negative bacteria: a number of Gram-positive bacteria have been shown to employ small, modified oligopeptides

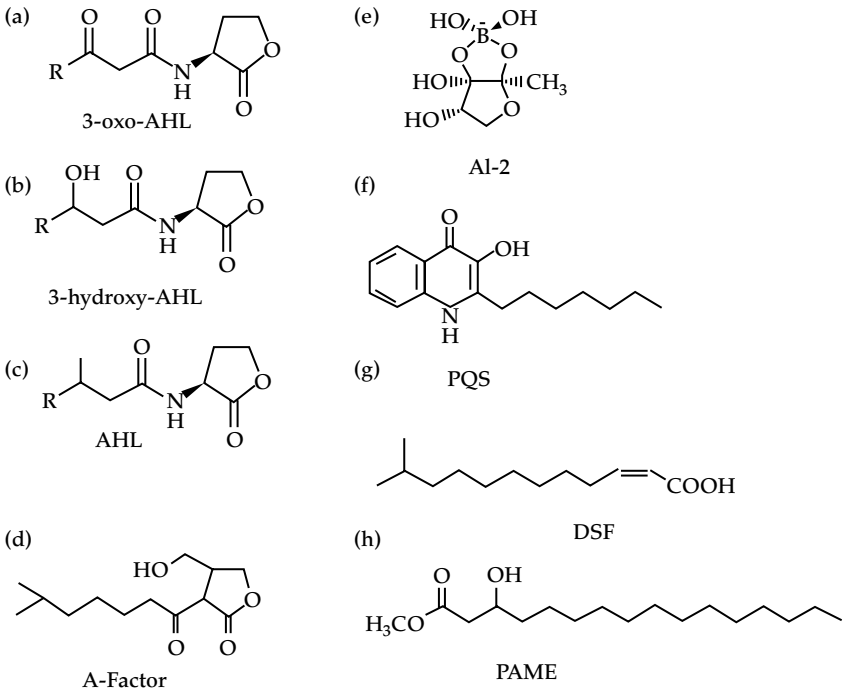

Figure 2.2 Structures of quorum sensing signal molecules found in bacteria. (a) 3-oxo-AHL; (b) 3-hydroxy-AHL; (c) *N*-acyl homoserine lactone (R ranges from C1-C15); (d) A-factor (2-isocapryloyl-3-hydroxy-methyl-γ-butyrolactone; (e) AI-2 (autoinducer-2); (f) The Pseudomonas quinolone signal (PQS, 2-heptyl-3-hydroxy-4(1*H*)-quinolone); (g) DSF (diffusible factor, methyl dodecenoic acid); (h) PAME (hydroxyl-palmitic acid methyl ester).

as extracellular signalling molecules. These peptides activate gene expression by interacting with two-component histidine protein kinase signal transduction systems (Kleerebezem *et al.* 1997). For example, in *Staphylococcus aureus* the expression of a number of cell density-dependent virulence factors is regulated by the global regulatory locus *agr* (accessory gene regulator) (Williams *et al.* 2007).

2.4 Evolutionary problems of signalling and cooperation

2.4.1 The problems of communication and cooperation

Two problems that have received much attention in the field of evolutionary biology are cooperation and communication (Hamilton 1964; Maynard Smith and Harper 2003), and these two issues come together in QS (Brown and Johnstone 2001; Redfield 2002; Keller and Surette 2006; Diggle *et al.* 2007b). In this section we consider the conditions under which QS to coordinate cooperation can be evolutionarily stable. We base our review of the relevant theory on Diggle *et al.* (2007b).

The problem of cooperation is why should an individual carry out a cooperative behaviour that is costly to perform, but benefits other individuals or the local group (Hamilton 1964). Such cooperation is vulnerable to invasion by cheaters who do not cooperate, but gain the benefit from the cooperation of others. This problem is well known in the fields of economics and human morality, where it is termed the tragedy of the commons (Hardin 1968). The tragedy is that, as a group, individuals would do better with cooperation, but this is not stable because each individual gains by selfishly pursuing its own short-term interests.

We have recently reviewed this problem in a microbial context elsewhere (West *et al.* 2006, 2007a). An obvious case in which it arises is when cells produce extracellular products for nutrient acquisition (Dinges *et al.* 2000; Greig and Travisano 2004; Griffin *et al.* 2004), antibiotics (Riley and Wertz 2002), immune modulation molecules (Brown 1999; Tateda *et al.* 2003; Hooi *et al.* 2004), antibiotic degradation compounds (e.g. β-lactamases) (Ciofu *et al.* 2000), and bio-surfactants (e.g. rhamnolipids) for motility (Velicer and Yu 2003; Daniels *et al.* 2004). These products are costly to an individual to produce, but provide a benefit to the individuals in the local group or population. Economic and evolutionary theory refers to such things as public goods (Dionisio and Gordo 2006). Many bacterial products termed 'virulence factors' are likely to be public goods—their coordinated production leading to damage to the host. The problem in these cases is that cheaters who do not pay the cost of producing such goods can still gain the benefit from neighbouring cooperators who do (for an experimental demonstration see Griffin *et al.* (2004) and Diggle *et al.* (2007c). This makes the cooperative production of public goods unstable, unless a mechanism such as kin selection operates (see below) (West and Buckling 2003).

The problem of communication is how can communication be reliable (Maynard Smith and Harper 2003)? Why do individuals convey honest information about themselves, to the benefit of other individuals? Why would they not give a false signal to their selfish advantage? If communication isn't reliable, then why should the receiver listen to it? The problem is reviewed for communication in general by Maynard Smith and Harper (2003) and within the specific context of bacteria by Keller and Surette (2006) (see also Chapter 1).

2.4.2 The problem of quorum sensing

Quorum sensing is generally assumed to coordinate cooperative behaviours in bacteria. Specifically, QS appears to provide a means for individual bacteria to assess local cell density and to engage in cooperation once a threshold density has been reached. Many cooperative ventures will not be worthwhile until a sufficient number of cells are present, so one would expect facultative cooperation based on the presence of cues such as QS molecules that act as a proxy for cell density. The idea is that signalling molecules are released, and that this rate of release is further increased by signalling molecules. This leads to positive feedback at high cell densities, and a dramatic increase in cooperative effort (Diggle *et al.* 2007a; Williams *et al.* 2007). (See Chapter11 for a related discussion on collective behaviours in other taxa.)

However, this communication may potentially be invaded by cheats that exploit this system (Brown and Johnstone 2001; Redfield 2002; Keller and Surette 2006). One possibility is a cheat that does not produce QS molecules (signal negative), and so benefits from monitoring the local cell density without investing effort into the dissemination of this information. An alternative possibility would be for a cheat to neither make the costly signal nor to respond to it (signal blind). A further possibility is for a signal blind cheat to make a signal but not respond. The crucial point here is that both signalling and responding to a signal with the production of public goods are costly. Consequently, there must be benefits that outweigh these—otherwise the system could be invaded by cheats that did not signal or cooperate.

As has previously been discussed, there are many species of bacteria that use QS to regulate the production of public goods and are therefore exploitable by cheats. It is important to note that many *P. aeruginosa* clinical isolates are 'signal blind' (i.e. they may or may not make minimal amounts of signal but, importantly, do not respond to a signal) (Denervaud *et al.* 2004; Smith *et al.* 2006), and so it is desirable to understand the costs and benefits of QS from an empirical perspective. A fundamental first step is to determine the **fitness** consequences of producing and responding to a signal. Calculating the number of ATP molecules required to make signal, Keller and Surette (2006) suggested that the cost of production of QS molecules varies from low to high depending on the type of signal molecule produced (Keller and Surette 2006).

Whilst there is undoubtedly a cost in making a signal, it is likely that the cost of responding is more metabolically expensive, especially when you consider that 6% of the *P. aeruginosa* genome changes in response to the addition of QS molecules. Given high costs, QS signalling or response could be potentially exploitable by QS cheats (Keller and Surette 2006; Diggle *et al.* 2007b). In theory, QS cheats could take the form of either: (1) a 'signal negative' strain which does not make the molecule but can respond to it, or (2) a 'signal blind' strain

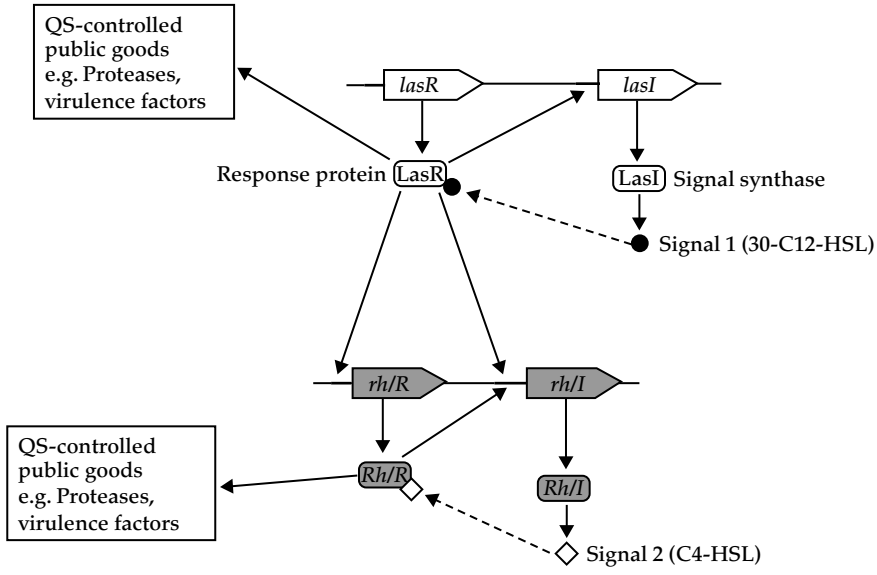

Figure 2.3 The hierarchical quorum sensing (QS) system of *P. aeruginosa*. The QS cascade is induced at high population cell densities when within the cell, the LasR response protein binds to a critical concentration of 3O-C12-HSL signal which has been produced by neighboring cells and taken up from the surrounding environment. This results in activation of the *las* QS system and the production of a number of QS-regulated public goods such as the proteases. Activation of the *las* system is also important in the induction of the *rhl* QS system which is also required for the production of proteases and a number of other *rhl*-controlled public goods.

which may (or may not) make signal but, more importantly, does not respond to it.

Recently we have been addressing empirically (using *P. aeruginosa*) whether QS is costly and subject to cheating behaviour (Diggle *et al.* 2007c). In *P. aeruginosa*, QS is controlled by two pathways (homologous to the *V. fischeri luxIR* system) which regulate the production of AHL signalling molecules (Fig. 2.3). These two systems are termed *las* and *rhl*, and use different AHL signal molecules, synthesized via LasI [*N*-(3-oxododecanoyl)-homoserine lactone (3O-C12-HSL)], and RhlI [*N*-butanoylhomoserine lactone (C4-HSL)], respectively (Latifi *et al.* 1995, 1996; Winson *et al.* 1995). Importantly, in *P. aeruginosa* QS regulates many potential social traits such as virulence, biofilm formation, and swarming motility. To examine the consequences of QS for social fitness, we focused on the *las* QS pathway because this system is top of the QS hierarchy (Fig. 2.3) (Latifi *et al.* 1996; Pesci *et al.* 1997), and a mutation in the *las* system results in the general abolition of QS.

We constructed both signal negative (*lasI*-) and signal blind (*lasR*-) mutants. Importantly, in the laboratory we can experimentally alter the level of signal perceived by either the wild type or the signal negative mutant by adding synthetic signal, which is chemically identical to that produced by *P. aeruginosa*, to cultures (Chhabra *et al.* 2003). We first examined the fitness consequences of QS in a situation where cooperation is favoured. A group of exoproducts whose production is controlled by QS in *P. aeruginosa* are the proteases. We examined the growth of the wild type and the signal negative and signal blind mutants in a medium where the ability to make proteases is required for growth. We found that: (1) both the signal negative and signal blind mutants grew very poorly in this medium when compared with the parental wild-type strain; (b) addition of synthetic signal to the signal negative strain significantly improved growth, as would be expected, because this will stimulate the production of proteases; (c) addition of signal to the signal blind strain resulted in no improvement in growth, as would be expected because the cells do not respond to the signal (Diggle *et al.* 2007c). This shows that QS can provide a benefit at the population level, by increasing the production of cooperative exoproducts, that can aid growth in certain environmental conditions.

We then determined whether the production of the QS signal molecules and cooperative QS-dependent exoproducts (public goods) is costly. We did this by comparing the growth rate of the mutants and the wild type in nutrient-rich Luria–Bertani (LB) broth, where the exoproducts produced by QS are not needed for growth. In these conditions, the QS mutants were able to grow to a higher density than the wild type. Addition of synthetic signal molecule to the signal negative mutant resulted in growth profiles similar to those seen for the wild type, suggesting that the response to QS signal molecules is costly as similar results were not seen when signal was added to the signal blind strain. These results suggests that upon entry to the stationary phase, QS signalling and the production of QS-dependent public goods place a heavy metabolic load on the cell (Diggle *et al.* 2007c).

Thus, it can be shown experimentally that QS is a social trait susceptible to exploitation and invasion by cheats. Given this, how is QS maintained in natural populations? The most likely explanation is kin selection, with cooperation being favoured because it is between close relatives.

2.4.3 A kin selection model of quorum sensing

Kin selection theory provides an explanation for cooperation or communication between relatives (Hamilton 1964). By helping a close relative reproduce, an individual is still passing on its own genes to the next generation, albeit indirectly. This theory is formalized by **Hamilton's rule** (Hamilton 1964), which states that **altruistic** cooperation is favoured when $rb - c > 0$; where c is the fitness cost to the altruist, b is the fitness cost to the beneficiary, and r is their genetic relatedness. This predicts that individuals should be more likely to cooperate when social partners are more closely related (higher r). For example, high levels of production of public goods are predicted when relatedness is higher among interacting bacteria (West and Buckling 2003). Relatedness can often be extremely high in bacteria because limited dispersal and clonal reproduction can lead to the individuals interacting

over a small area being predominantly clone-mates (West *et al.* 2006).

Brown and Johnstone (2001) developed a kin selection model of QS. They assumed:

1. Signalling is costly to the individual. The fitness of an individual cell is negatively correlated to the amount of signalling by that individual.
2. The production of public goods, in response to QS, is costly to the individual. The fitness of an individual cell is negatively correlated to the amount of public goods produced by that individual.
3. The production of public goods provides a benefit to the local group of interacting cells (the group). The fitness of an individual cell is positively correlated to the average amount of public goods produced by the local individuals.
4. The benefit of producing public goods is greater at higher population densities. The fitness benefit to an individual cell of a certain level of local production of public goods is positively correlated with cell density.

Brown and Johnstone (2001) then made predictions for the evolutionarily stable level of signalling (production of signalling molecule) and public goods production (cooperation). A behaviour is described as an **evolutionarily stable strategy** (ESS) if it cannot be invaded or beaten by a mutant performing any other strategy once it has been adopted by the majority of individuals (Maynard Smith and Price 1973). In particular, they examined the consequences of variation in mean population density and relatedness (r). They found that:

1. *Result 1.* The ESS level of signalling and public goods production both increased with greater population densities. At low densities there is little to be gained from the cooperative production of public goods.
2. *Result 2.* The ESS level of production of public goods increased with higher relatedness between interacting bacteria (Fig. 2.4a). This is expected because greater levels of cooperation are favoured with a higher relatedness. However, appreciable levels of cooperation can be predicted even when relatedness is relatively low.
3. *Result 3.* The ESS level of signalling showed a domed relationship with relatedness (Fig. 2.4b). At high relatedness there is a shared interest in cooperation, and in cheap signalling. At low relatedness, there is no selection for cooperation, and hence no selection for signalling to coordinate this. With intermediate relatedness, there can still be selection to produce public goods, but it is in the individual's interest to produce fewer public goods than the other local cells (because $r < 1$). This favours higher levels of signalling in an attempt to manipulate competitors to cooperate more (which in turn leads to the signal being increasingly ignored). This is termed 'competitive devaluation of signal strength' (Brown and Johnstone 2001).

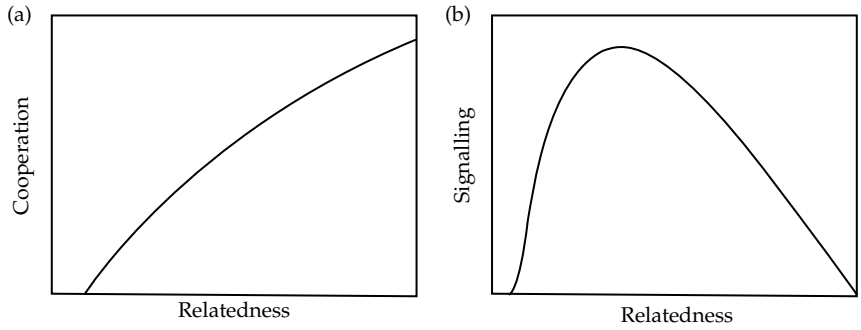

Figure 2.4 Brown and Johnstone's theoretical model of quorum signalling. (a) Cooperation effort increases with increasing relatedness, because the inclusive fitness benefits of cooperation are maximal at high relatedness and minimal at low relatedness. (b) Signalling effort is a dome-shaped function of relatedness, because at low relatedness there is little inclusive fitness benefit to be accrued from organizing a cooperative venture, and at high relatedness there is little conflict so that a cheap signal is favoured, whereas at intermediate relatedness cooperation is worthwhile yet there is also scope for conflict so a costly signal is required to initiate competition.

Experimentally we tested Brown and Johnstone's theory that QS can be maintained by kin selection. Using a QS-positive wild type (QS positive) and a signal blind cheat, mixed together (1:1) in a medium where the ability to quorum sense is essential for survival, we found that QS was favoured at a relatively high relatedness. This is in agreement with Brown and Johnstone's prediction that cooperation would increase with higher relatedness (Fig. 2.4a). Under conditions of high relatedness, and a number of rounds of selection, the wild-type cells constituted 100% of the total population. In contrast, in conditions of low relatedness, the cheats increased in frequency to approximately 60% after a number of rounds of selection. Therefore, low relatedness within a population allows cheats who do not quorum sense to exploit the individuals who do (Diggle *et al.* 2007c).

2.4.4 Other models of quorum sensing

Brown and Johnstone's (2001) model provides a clear and elegant application of kin selection theory to QS. However, as they stress, it makes many simplifications, the relaxing of which may have important consequences. Furthermore, much more has been learnt about QS since, and we should also consider alternative possible explanations for QS.

Brown and Johnstone's (2001) model could be extended to investigate the consequences of several biological complexities. It has been found that signalling molecules can have multiple functions, and this would alter the relative cost and benefit of their production, as well as how this would vary with the social context. For example, they can also function as antibiotics (Stein 2005), potentially as public goods such as iron-scavenging molecules (Kaufmann *et al.* 2005; Diggle *et al.* 2007d), and as potent immune modulators (Tateda *et al.* 2003; Hooi *et al.* 2004). Production and secretion of signal molecules may also be linked to the production of other molecules through excretion in membrane vesicles (Mashburn and Whiteley 2005). Another possibility is that different types of signal need to be considered, with different costs or specificities. It appears that specificity and cost vary across signals, with cheap-to-produce signals being utilized very generally across species, and more expensive signals being more specific, within species, possibly even within lineages (Keller and Surette 2006).

Kin selection is not the only possible explanation for cooperation (Sachs *et al.* 2004; see an individual-level hypothesis by Zahavi in Chapter1) An alternative explanation for cooperation is that it provides a direct benefit to the individual performing the behaviour, which outweighs the cost of performing the behaviour (i.e. it is mutualistic not altruistic). An example of this would be if the waste product of one species provided a benefit to individuals of a second species (by-product benefit), and hence the second species could be selected to cooperatively help individuals of the first species, in order to increase the by-product benefits (Sachs *et al.* 2004). It would be extremely interesting to see whether communication between species can be evolutionarily stable in such cases. There are several other forms of direct benefit to cooperation that could be examined from a QS and communication perspective—for example, when cooperation is stabilized between non-relatives by policing or punishment of non-cooperators (Frank 2003).

2.5 Defining signalling in bacteria

As discussed earlier, the fact that a compound produced by cell A elicits a response in cell B does not necessarily mean that there is true signalling between the cells and may represent cell B using the molecule as a 'cue' or cell A coercing cell B into a certain action. In this section we discuss examples of QS between single populations and mixed populations of bacteria and suggest whether this can be considered signalling, a response to a cue, or a coercion (see also Keller and Surette 2006).

In general, communication in bacteria can be divided into three main areas:

1. Intraspecies: communication arising or occurring within a single bacterial species.
2. Interspecies: communication arising between two or more distinct species of bacteria.
3. Interkingdom: communication arising between a bacterial species and a higher organism.

2.5.1 Intraspecies communication

In Gram-negative bacteria, the most intensely studied QS systems rely upon the interaction of AHL signal molecules synthesized by LuxI-type AHL synthases, with LuxR-type transcriptional regulator proteins (see Section 2.3). A simple example of this can be seen in the marine bacterium *V. fischeri* (Nealson *et al.* 1970). This organism forms a symbiotic relationship with the squid *Euprymna scolopes* where it colonizes the light organ (McFall-Ngai and Ruby 2000). At low cell densities the bacterial population does not luminesce but at high densities there is a coordinated switch on of bioluminescence. This production of light has been shown to be mediated by a diffusible AHL molecule (3O-C6-HSL) synthesized by the LuxI protein. At a critical concentration, 3O-C6-HSL binds to LuxR and the complex activates expression of the *luxCDABE* operon resulting in coordinated production of bioluminescence. Under laboratory conditions, it is possible to stimulate early induction of bioluminescence simply by providing the cells with exogenous 3O-C6-HSL. It is not entirely clear why *V. fischeri* cells have a shared interest that favours signalling and cooperation to produce light. Possibilities are a high relatedness between the cells within a light organ, or the avoidance of punishment from the host squid if light is not produced (analogous to why rhizobia fix nitrogen for their host plants (West *et al.* 2002a; Kiers *et al.* 2003)). Indeed, it appears to be the case that the squid can enforce bioluminescence by altering the environment such that *lux*-deficient strains are defected in light organ colonization. It was hypothesized that a diminished level of oxygen consumption by *lux*-deficient strains is responsible for the reduced fitness (Visick *et al.* 2000).

As many species of Gram-negative bacteria have been shown to produce AHL signalling molecules, then similar examples can be seen in other species (Diggle *et al.* 2007a; Williams *et al.* 2007). Some bacteria have been shown to regulate the production of virulence determinants in a cell density-dependent manner. For example, *Erwinia carotovora* subsp. *carotovora* coordinately produces both exoenzymes, which destroy plant tissue, and the antibiotic carbapenem in response to critical concentrations of 3O-C6-HSL (Jones *et al.* 1993). Similarly the opportunistic pathogen *P. aeruginosa* regulates an arsenal of extracellular virulence factors using a complex hierarchical QS cascade involving two major AHL molecules, namely 3O-C12-HSL and C4-HSL (Venturi 2006). In such cases it is likely that these are examples where QS molecules can be classed as 'signals' between cells as the production by cell A has evolved due to its effects on cell B which in turn has evolved a response to the signal (Maynard Smith and Harper 2003). We suspect that kin selection is the mechanism to explain the evolutionary stability of such signalling, as discussed in Section 2.4. Although the AHL family of QS molecules have been described in a wide variety of Gram-negative bacterial species (Lazdunski *et al.* 2004), crucially they tend to differ between bacterial species. AHLs consist of a conserved homoserine lactone ring connected via an amide bond to an acyl side chain which can vary in length from 4 to 18 carbons. In addition, these side chains may or may not be modified with a 3-hydroxy or a 3-oxo group, potentially providing a large variety of AHL molecules. Many species of bacteria will only respond to their cognate molecule(s) providing a certain degree of specificity, and therefore AHL signalling is generally of an intraspecies nature. Some bacteria, however, are able to 'exploit' AHLs produced by another species, and this will be discussed later.

Whilst it is plausible to view AHLs as signals between cells of the same species, the situation is often more complicated as some AHLs have been shown to have multiple functions. For example 3O-C12-HSL produced by *P. aeruginosa* has been reported to have immunomodulatory properties (Telford *et al.* 1998; Tateda *et al.* 2003). It is unlikely that this involves signalling between the host and bacteria. More likely, this represents 3O-C12-HSL 'chemically manipulating' or 'coercing' the host immune response to the benefit of the bacterial population.

The world of microbial communication is not limited to Gram-negative bacteria. Gram-positive bacteria also produce QS molecules but tend to utilize post-translationally modified autoinducing peptides (AIPs). For example, *S. aureus* uses AIPs to regulate the production of exotoxins in response to a critical concentration of peptide (Novick 2003).

Explaining within-species cooperative signalling requires some kind of mechanism (see also Chapter11). The production of a costly signal for the common good makes this type of communication exploitable by cheats who do not contribute to signal production but reap the benefits of QS-mediated behaviour, for example acquisition of nutrients provided by QS-dependent exoenzyme production. In fact, recent work has shown that many *P. aeruginosa* clinical isolates are QS defective and make very few virulence factors when grown in the laboratory (Denervaud *et al.* 2004; Schaber *et al.* 2004; Lee *et al.* 2005) suggesting that it may be beneficial not to signal under certain environmental conditions, or that cheats can invade in long-term infections (West *et al.* 2006). As local populations of cells are likely to be closely related, then one way that cooperation can be maintained is via kin selection, which requires a sufficiently high relatedness between cooperating individuals (West *et al.* 2006). Limited dispersal (population viscosity) would tend to keep relatives together (Hamilton 1964). In this case, indiscriminate altruism may be favoured because neighbours will tend to be relatives (Hamilton 1964; Queller 1992; West *et al.* 2002b). This type of mechanism is likely to be of huge importance in microorganisms where asexual reproduction means that single cells colonize and grow in a local area. In this case, the individuals interacting over a small area will be clonal, which can be very conducive to the evolution of cooperation.

2.5.2 Interspecies communication—bacterial 'cross-talk'

A third class of QS signal molecule has been described in the marine bacterium *Vibrio harveyi*. Bioluminescence in this organism is cooperatively regulated by AHLs and a molecule termed autoinducer-2 (AI-2) which is a furanosyl borate diester produced by the enzyme LuxS (Chen *et al.* 2002). The identification of the *luxS* gene required for the production of AI-2 production (Surette *et al.* 1999) sparked an exponential increase in AI-2 signalling research. The reason being that the *luxS* gene can be found in a wide variety of bacterial genera (Winzer *et al.* 2002, 2003).

Importantly, representatives of both Gram-negative and Gram-positive bacteria carry this particular gene, and consequently AI-2 production has been demonstrated in many species of bacteria. This has led to the hypothesis that AI-2 is employed as a means of interspecific communication or 'bacterial Esperanto' (Winans 2002). This idea is difficult to explain from an evolutionary point of view, as cooperation between species is even harder to explain than within species. The major difference is that kin selection, as discussed in Section 2.3, will not be important across species. There are mechanisms by which cooperation can be favoured between species, such as by-product benefit (Sachs *et al.* 2004), or to avoid punishment (West *et al.* 2002a; Kiers *et al.* 2003), but these are expected to be rarer (West *et al.* 2006).

It must therefore be questioned whether AI-2 can be defined as a true signal. For this to be the case AI-2 must: (1) be diffused from the cell, (2) be taken up by a neighbouring cell, (3) elicit a response from that cell because the receiver's response has evolved, (4) benefit both producer and receiver. Clearly points 1 and 2 are met with respect to AI-2 but there are major doubts about points 3 and 4. Despite AI-2 being produced by many genera, there is very little evidence linking it with direct activation of any specific genes. Studies in many different bacteria have shown that *luxS* mutants differ phenotypically from wild-type strains; however, this can often be explained because of a defect in a metabolic pathway. It is now well known that LuxS plays an important role in bacterial metabolism, contributing to the recycling of *S*-adenosyl-L-methionine (SAM), of which AI-2 is a metabolic by-product (Winzer *et al.* 2003). To date only bioluminescence in *V. harveyi* (Surette *et al.* 1999), and an ABC transporter in *Salmonella typhimurium* (termed Lsr) (Taga *et al.* 2001) have been shown to be regulated by AI-2. In these species, we can speculate that AI-2 may be used as a cooperative signal in an intraspecies context. Theoretically, these species could also use AI-2 from other organisms to regulate these respective traits. In this case, however, it is inaccurate to use the term interspecies signalling as the receiver's response has not evolved in parallel with the producing bacterial species. In this scenario we can say that both *V. harveyi* and

S. typhimurium use the metabolic by-product AI-2 as an environmental 'cue' to regulate gene expression. Interspecies signalling has also been suggested between avirulent oropharyngeal flora (OF) (AI-2 +ve) and *P. aeruginosa* (*luxS* and AI-2 –ve) within the cystic fibrosis (CF) lung (Duan *et al.* 2003). Co-incubation of *P. aeruginosa* with OF bacteria resulted in an increase in virulence gene expression which was attributed, at least in part, to AI-2. The mechanism for this is unknown as *P. aeruginosa* does not make AI-2 but we suggest that this is not an example of interspecies signalling. It is more likely that *P. aeruginosa* is able to use AI-2 as a cue, perhaps to assess its surroundings, or it may be that OF bacteria 'coerce' or manipulate *P. aeruginosa* into increased virulence which may provide them with more nutrients.

Interspecies signalling between bacterial species using AHL molecules has also been suggested. *Pseudomonas aeruginosa* and *Burkholderia cepacia* often occur together in the lungs of people with cystic fibrosis, where they are associated with high morbidity and mortality (Eberl and Tummler 2004; Govan and Deretic 1996). *Burkholderia cepacia* has been shown to up-regulate the production of virulence determinants in response to AHLs produced by *P. aeruginosa*, although this does not appear to happen the other way round. This type of behaviour has also been termed 'bacterial cross-talk' which is suggestive of a cooperative venture between two or more species. In this case, it suggests that *B. cepacia* uses *P. aeruginosa* AHLs as a cue to alter its behaviour rather than there being signalling between the two bacterial species. *Pseudomonas aeruginosa* pays the cost of producing AHLs, possibly for within-species signalling, but appears to gain no benefit from *B. cepacia* in return.

2.5.3 Interkingdom communication across the prokaryote/eukaryote divide

Several recent reports have demonstrated that bacterial QS molecules (specifically AHLs) can affect gene expression in eukaryotes as many eukaryotic **hormones** structurally resemble AHLs. Generally this has been termed interkingdom signalling or global sensing (Shiner *et al.* 2005). AHL molecules have been experimentally demonstrated to affect a number of animal cell types including murine and human primary cells (Telford *et al.* 1998), breast cancer cells (Li *et al.* 2004), bone marrow macrophages (Tateda *et al.* 2003), and primary porcine arterial smooth muscle cells (Lawrence *et al.* 1999). In addition, plant behaviour has also been shown to be modified by AHLs. The zoospores of the seaweed *Enteromorpha* have been shown to settle preferentially on AHL-producing biofilms of the marine bacterium *Vibrio anguillarum* (Joint *et al.* 2002). Furthermore, higher organisms have mechanisms that appear to downregulate QS in microorganisms. For example, the marine red alga *Delisea pulchra* produces a halogenated furanone that disrupts QS in several species of bacteria including the swarming motility of *Serratia liquefaciens* (Givskov *et al.* 1996). This furanone has also been shown to disrupt *P. aeruginosa* biofilms (Hentzer *et al.* 2002). These AHL 'mimics' attract interest as possible alternatives to antibiotic therapy. Whether these examples demonstrate signalling using small molecules between prokaryotes and eukaroytes is open to debate. In general, studies performed to date appear to show that either (1) the signalling bacterium manipulates or coerces its host into a certain action rather than there being a truly evolved signalling system between the two (cf. coercion strategies, Chapters 4 and 10) or (2) as in the example of the zoospore settlement, the eukaryote utilizes bacterial AHLs as an environmental cue as a guide to future action.

2.6 Complexities of bacterial communication

In agreement with behavioural studies on organisms such as birds, mammals, and insects, signalling in bacteria has a number of complexities that offer problems from an evolutionary perspective.

First, the signal can be degraded (as also occurs for other modalities such as sound and **pheromones**). This degradation can be environmental in nature or due to the action of certain enzymes. This signal interference has often been suggested as a possible way of controlling the virulence of pathogenic bacterial species (i.e. breaking the lines of communication) and thus leading to novel therapies. AHL signals are rendered biologically inactive in

alkaline environments (Yates *et al.* 2002) and therefore, in certain environmental niches, signalling may be ineffective. In theory, the levels of QS signalling may be greatly influenced by environmental conditions but whether this alters the cost and benefit of either making a signal, or responding, has not been explored. AHLs can also be degraded by enzymes produced by bacteria, a process known as quorum quenching (Dong and Zhang 2005). Examples include AiiA, an AHL lactonase produced by a *Bacillus* spp. (Dong *et al.* 2001), and PvdQ, an AHL-acylase produced by *P. aeruginosa* (Sio *et al.* 2006). This raises many interesting questions, which could be empirically tested. What effect can an AHL-degrading species have on an AHL producer? For instance, does degradation interfere with key social behaviours such as population swarming or result in the reduction of a number of harmful AHL-dependent exoproducts which is ultimately beneficial to the degrading organism? Can this behaviour be considered coercive or spiteful, and are there indirect or direct fitness benefits for the AHL degrader? Is AHL degradation evolutionary stable or is it subject to invasion by cheats who do not make the degrading enzymes?

Second, the genes required for signal generation (*luxI* homologues) and response (*luxR* homologues) are not always found on the bacterial chromosome. A number of these homologues have been identified on plasmids such as the *Agrobacterium* Ti plasmid (Zhang *et al.* 1993) and *Rhizobium* symbiotic plasmids (Smith 2001; Wisniewski-Dye and Downie 2002). While this may just represent an easy way to obtain QS mechanisms, could it also be a mechanism by which signalling is forced onto a cell that doesn't contain the QS machinery, coercing it into cooperative behaviour? An important point here is the **conflicting** interests of the bacteria involved, and the plasmids themselves. Third, QS molecules are not just signals. A number of other roles have been assigned to QS molecules which suggests they can also function as public goods, for example iron chelators (Diggle *et al.* 2007d), immunomodulatory compounds(Pritchard 2006), and biosurfactants (Daniels *et al.* 2006). QS compounds can also be harmful or spiteful, for example the AIP lantibiotics typified by lactococcal nisin and produced by *Lactococcus lactis* are potent bacteriocides against many Gram-positive organisms (Stein 2005). The consequences of QS signals having multiple functions needs to be explored theoretically (Brown and Johnstone 2001; Diggle *et al.* 2007b).

Another complexity of studying signalling in bacteria is that most bacterial species are capable of forming structured multicellular communities known as biofilms (Kolter and Greenberg 2006). Biofilms are ubiquitous, being found in such diverse environments as dental plaques, wounds, rock surfaces, and at the bottom of rivers. They have a definite structure, including water channels, which may involve a number of different 'specialist' cells and they are often enclosed by a exopolysaccharide matrix which can make them difficult to eradicate. It is also comparatively harder to empirically study cells growing in a biofilm compared with planktonic cells. However, biofilms are of particular interest from an evolutionary perspective, because the close proximity of individuals in a biofilm can make cooperation and communication particularly important.

Many forms of cooperation can be involved in the establishment and growth of a biofilm, such as the cooperative production of an extracellular matrix which surrounds the biofilm, and may be important in maintaining structure (Davies and Geesey 1995; Nivens *et al.* 2001; Friedman and Kolter 2004; Matsukawa and Greenberg 2004; Diggle *et al.* 2006b). In addition, numerous other public goods can be important in biofilms, such as rhamnolipid, a biosurfactant which aids in biofilm detachment (Boles *et al.* 2005), and microvesicles which are a component of the extracellular matrix and can contain signal molecules and proteases (Schooling and Beveridge 2006). Quorum sensing may play an important role in the development and structuring of biofilms produced by certain bacterial species, as suggested by the poor biofilm formation of some QS mutants (Davies *et al.* 1998), although, perhaps surprisingly, not a great deal is known generally about QS and biofilm development which may stem from the fact that biofilms are difficult to study experimentally. However, it has been shown in *P. aeruginosa* that QS plays a role in biofilm differentiation (Fig. 2.5).

The evolutionary implications of QS in biofilms are also uncertain. It could be expected that kin

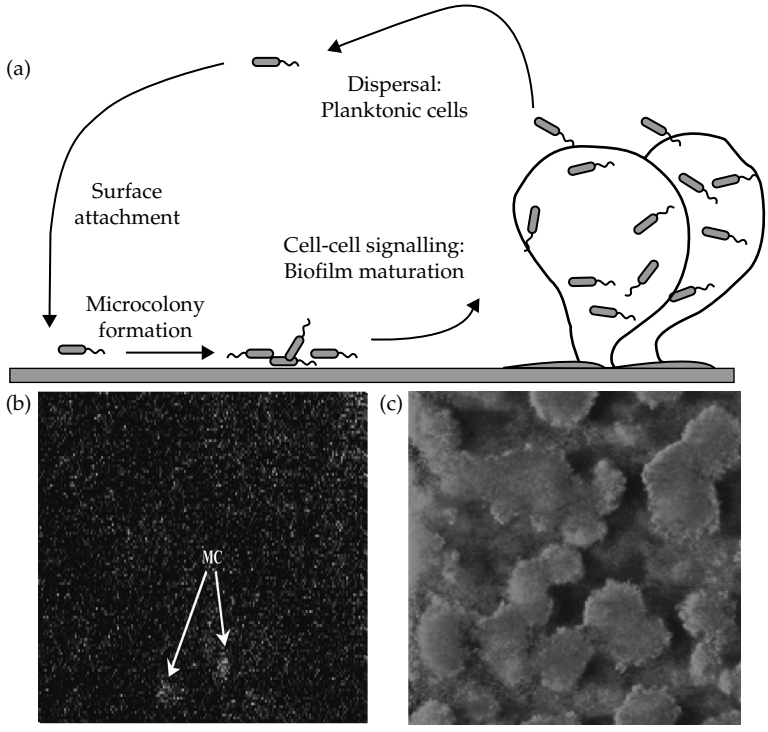

Figure 2.5 Lifecycle of a bacterial biofilm. (a) Planktonic cells are released from mature biofilms, and via motility mechanisms they settle on a new surface. Cells become irreversibly attached and begin to form microcolonies. Mechanisms such as cell-cell signalling systems lead to the differentiation of mature biofilm structures. Diagram adapted from (Kolter and Losick 1998). (b) Scanning electron microscopy image of *P. aeruginosa* attachment to stainless steel coupon. The formation of microcolonies can be observed (MC), image taken from (Diggle *et al.* 2006b). (c) Scanning confocal microscopy image of mature 5 day old *P. aeruginosa* biofilms grown in flow cell chambers. Image courtesy of S. Crusz.

selection is important in biofilms initiated by one or a small number of clonal lineages. However, naturally formed biofilms very rarely contain just one species of bacteria, let alone a single clonal lineage. For example, the colonization of human teeth and the oral mucosa can involve up to 500 species of bacteria (Kolenbrander *et al.* 2002). Nonetheless, kin selection may still be important in such cases if social interactions take place on a local scale. For example, if the benefit of producing the materials that structure the biofilm, such as exopolysaccharides, or other public goods (perhaps regulated by QS), are shared primarily with neighbouring cells, then the clonal growth of bacteria means that these benefits can still be shared with close relatives (Xavier and Foster 2007). In this case it might be useful to think of biofilms as consisting of a number of clonal lineages (groups of lineages), with cooperation primarily within lineages but competition primarily between lineages.

Some workers suggest that QS is not true cell-to-cell signalling and that is an artefact of laboratory conditions. Redfield (2002), has argued that autoinducer molecules are not released to signal to other cells. Redfield suggests that autoinducer secretion and response may have a more direct benefit, by allowing individual cells to determine how rapidly secreted molecules move away from the cell. This diffusion sensing (DS) could allow cells to regulate secretion of costly public goods to minimize losses owing to extracellular diffusion and mixing. This is an alternative explanation

for the evolution of QS, and diffusion effects could also be incorporated into kin selection models. It is important to consider how QS and DS may have evolved. In the case of QS, it may be assumed that it evolved because of group benefits, but when you consider DS this is likely to have evolved because of benefits to the individual. It should also be considered that production of these molecules may have initially evolved for one reason (e.g. DS), but is now maintained for another (e.g. QS). Hense *et al*. (2007) recently introduced a new term: efficiency sensing (ES) which unifies the QS and DS theories. Efficiency sensing argues that sensing will have been favoured by both individual and group benefits as the cells measure a combination of cell density, mass-transfer properties, and spatial cell distribution (Hense *et al*. 2007). The hypotheses need not be alternatives, as it may be the case that benefits of DS are crucial for the maintenance of this trait, yet they are still monitored for QS purposes. It is likely that both functions will be of importance in understanding when and why these molecules are produced.

2.7 Conclusions and future perspectives

Quorum sensing systems are widespread amongst Gram-negative and Gram-positive bacterial species. However, when one compares the microbiological literature on QS with the animal literature it is sometimes questionable as to whether QS in bacteria should always be regarded as signalling. It is likely that QS within species represents signalling, because the natural history of many microorganisms means that the interactions will be between close relatives and therefore cooperation and signalling can be explained by kin selection. However, QS is often described between species or even across kingdoms, and in these cases QS signals may be used as chemical cues or as coercion molecules. It is possible that these interactions may be due to signalling, but cooperation between species requires special conditions that are only rarely met. There is therefore increasing uncertainty as to what form of communication QS represents in bacteria and the challenges for the future will rely on experimental studies that examine the costs and benefits of communication to both the sender and responder. Furthermore, cooperation and communication need to be expanded empirically into biofilms as this is the natural state of growth for many species of bacteria. It is important to note that in one particular environment where biofilms are formed (the cystic fibrosis lung), *P. aeruginosa* QS signal blind mutants are often isolated; the reasons for this are poorly understood. Understanding the interactions between strains found within such environments will provide unique insights into eradicating problematic organisms such as *P. aeruginosa.*

Summary

The term quorum sensing (QS) is used to describe communication between bacterial cells, whereby a coordinated population response is controlled by diffusible signal molecules. Quorum sensing has not only been described between cells of the same species (intraspecies), but also between bacterial species (interspecies), and between bacteria and higher organisms (interkingdom). Here we compare the evolutionary literature on animal signalling and cooperation with the microbiological literature on QS, and discuss whether bacterial QS can be considered true signalling. From an evolutionary perspective, intraspecies signalling can be explained using models such as kin selection, but explanations become more difficult when communication is described between species. It is likely that this often involves QS molecules being used as 'cues' by other species as a guide to future action or as coercing molecules whereby one species will 'coerce' another into a response.

Acknowledgements

We would like to gratefully acknowledge the Royal Society as S.P.D., S.A.W., A.G., and A.S.G. are all supported by Royal Society Fellowships.

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