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Bacterial quorum sensing: the progress and promise of an emerging research area

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Preface

This review highlights how we can build upon the relatively new and rapidly developing field of bacterial communication or quorum sensing (QS). We now have a depth of knowledge about how bacteria use QS signals to communicate with each other and coordinate activities. There have been extraordinary advances in QS genetics, genomics, biochemistry, and diversity of signaling systems. We are beginning to understand the connections between QS and bacterial sociality. This foundation places us at the precipice of a new era where researchers can advance towards development of new medicines to treat devastating infectious diseases, and in parallel use bacteria to understand the biology of sociality.

Keywords

Sociomicrobiology; bacterial communication; *Vibrio*; *Pseudomonas aeruginosa*; orphan receptors; solo receptors; biogeography; quorum quenching; anti-virulence strategies

‘Competing is intense among humans, and within a group, selfish individuals always win. But in contests between groups, groups of altruists^a always beat groups of selfish individuals.’

E.O. Wilson

Main text

Humans have provided descriptions of the natural history of animals for millennia. Apart from basic anatomy and physiology, it was also noted that a number of animal species showed signs of “social intelligence”. Aristotle provided a description of animal social

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^aThe ethological definition of altruism is a behavior by an individual that increases fitness of another individual while decreasing fitness of the actor¹.

behavior in his 4th century BC book ‘History of Animals’, noting how ants march one after the other when putting away food, while bonitos swarm together when they catch sight of a dangerous creature. The application of evolutionary principles to social behaviors in the 19th century brought forth the field of sociobiology that was further explored and popularized in the 1970’s². While the last 40 years have been a time of great advancement and debate in sociobiology, these ideas were largely unexplored in the field of microbiology until the 21st century. We now know that microbes are highly gregarious communicating organisms and bacterial communication can modulate a range of behaviors important for fitness (reproductive success).

Background and a Brief History of Quorum Sensing

Bacterial QS involves self-produced extracellular chemical signals, which can accumulate in a local environment to levels that are required to activate transcription of specific genes^{3–5}. The first hints about QS came in the late 1960s and early 1970s, when investigators showed that genetic competence in *Streptococcus pneumoniae*⁶ and luminescence in two species of marine bacteria^{7,8} required production of extracellular molecules. Cell-cell signaling via these molecules was proposed as a form of chemical communication, but these early publications were met with skepticism and generally ignored for the next 10–20 years. The 1980s brought two landmark discoveries: (i) the luminescence (*lux*) genes from the marine bacterium *Vibrio fischeri* were identified, and the genes required for what is now called quorum control of luminescence, *luxI* and *luxR*, were shown to control *lux* gene transcription^{9,10}; and (ii) the QS signal from *V. fischeri* was determined to be *N*-3-oxohexanoyl-L-homoserine lactone (3OC6-HSL)¹¹ (Fig. 1A). The *luxI* gene codes for the autoinducer synthase required for 3OC6-HSL production, and *luxR* codes for a 3OC6-HSL-responsive transcriptional activator of the *lux* genes (Fig. 1A).

General interest remained muted for another decade. In the 1990s DNA sequencing and comparative sequence analysis became everyday laboratory procedures, and gene pairs with homology to *luxR* and *luxI* began to attract the curiosity of some investigators. This led to an explosion of findings that other bacterial species controlled genes for conjugation, exoenzyme production and antibiotic synthesis with LuxI-LuxR-like systems³. A common theme emerged; the LuxI homologs catalyzed synthesis of an acylated homoserine lactone (AHL) and the LuxR homologs all showed specificity for their cognate AHL. This convergence of discoveries led to the QS concept (Fig. 1A); that the diffusible AHLs served as a proxy for cell density and allowed a bacterial species to produce costly extracellular public goods only when there was a sufficient biomass to benefit from the public goods⁴. Shortly thereafter the QS signal from *S. pneumoniae* (often referred to as a pheromone) was shown to be a small peptide¹², and *Staphylococcus aureus* was shown to use small cyclic peptide pheromones to activate genes for production of extracellular toxins¹³. Quorum sensing was therefore shown to occur in Gram-positive and Gram-negative bacteria via diverse chemical signals (Fig. 1B). An early study showed that a luminescent marine bacterium called *Vibrio harveyi* could sense a self-produced signal and also a signal or signals produced by other bacterial species to induce light production⁷. This phenomenon is considered to be a type of QS, where cells of many species in a mixed microbial community sense the general bacterial population density via a molecule termed Autoinducer-2

(AI-2)^{14,15}. The strengths and weaknesses of this concept are discussed later in this review. Further studies also described QS-like systems in eukaryotic microbes (the pathogenic fungi *Candida* and *Histoplasma*)^{16,17} and very recently in viruses¹⁸, thus providing clear examples of convergent evolution. Some QS signals are volatile, for example the DSF and PAME signals shown in Figure 1, and there is some evidence that volatile signaling can occur in a local atmosphere¹⁹. Functional studies followed the discovery of many of these systems and revealed that for many plant and animal pathogens, QS mutants showed greatly reduced virulence^{13,20,21}. The early connection between QS and pathogen virulence brought forth excitement about the idea of targeting QS as a novel approach to treat bacterial infections (Box 1).

Box 1

The challenge to QS therapeutic development

Early discovery that QS mutants of important plant and human pathogens are attenuated for virulence^{20,21} led quickly to the concept of using QS inhibitors to control some diseases¹¹⁰. In fact, a variety of small molecule inhibitors of QS signal receptors and LuxI-type QS signal synthases have been discovered, as well as enzymes, which degrade AHL signals^{90–92,99–104}. Yet we face obstacles in moving from the bench to the clinic. Many of the obstacles are inherent to drug discovery such as lack of potency in animal models, delivery, toxicity, stability, and a narrow spectrum of activity. But what are other obstacles? Fundamental questions remain unanswered. At what point in an infection will QS inhibition be of value? Will QS inhibitors find general utility or will they function best as prophylactic agents? Can biological interference approaches be contemplated? Can we imagine introducing a benign bacterial species, which produces an AHL lactonase to interfere with a QS pathogen into a human? We note that such an anti-QS approach has been developed to control membrane fouling by biofilms in water purification plants (see image below)¹¹¹. What pathogens should we target? What are the regulatory hurdles for a QS inhibition therapeutic to even enter a clinical efficacy trial? How quickly will resistant bacteria emerge? There is an idea that because QS inhibitors are anti-virulence agents rather than antibacterial agents, resistance is less likely to emerge. Laboratory experiments do not support this idea⁹⁸ but in theory the route to the spread of resistance may be slow and depending on the resistance mechanism it may be self-limiting. The route to testing anti-QS approaches may be much less tortured in the case of crop diseases where any number of approaches can be easily tested in the laboratory or greenhouse. Ultimately, if QS inhibition is to gain therapeutic utility for human diseases, this will likely occur in the context of combination therapies with conventional antimicrobials.

**Box 1 image.**

Quorum-quenching membrane filtration (QQ-MBR) pilot plant system at sewage treatment facility in Seoul, South Korea. Professor Chung-Hak Lee of Seoul National University (center) has led a decade-long program to develop this technology

Sociomicrobiology: An evolutionary perspective on quorum sensing

Early research assumed that QS is a social trait (a trait that impacts both the individual performing a behavior and a recipient²²), but this concept was not tested experimentally. In principle, QS could be non-social, for instance allowing individual bacteria to sense their physical environment (this has been termed diffusion sensing)^{23,24}. However, there is now a large body of evidence showing that in certain environments, QS is social and that at the population level, QS regulates the production of extracellular public goods^{22,23,25–29}. Importantly, whilst public goods directly benefit the producing cell, they also indirectly socially benefit surrounding cells. Because the production of a public good is costly, these behaviors are potentially exploitable by non-producing ‘cheats’, creating bacterial social dilemmas (Fig. 2). QS has now been shown to be exploitable by cheats in laboratory cultures^{26–29}. One well-studied example is with the pathogenic bacterium *Pseudomonas aeruginosa*. This bacterium requires QS to induce production of extracellular proteases required for growth on proteins. QS mutants fail to grow by themselves on milk protein, but in co-culture with QS competent protease producing cells the mutants have a fitness advantage when at relatively low relative abundance^{25,26,29}. In fact mixed infections of cooperating and cheating cells have been shown to be less virulent than single strain cooperator infections³⁰. This has led to the idea of using bacterial cheats to help treat infections by reducing virulence or by introducing ‘Trojan Horse’ genes into virulent populations³¹.

Given that QS can be readily exploited by cheats, why are numerous functional QS systems maintained in natural populations? This was a dilemma first discussed by Darwin: if cheats in a population have a fitness advantage over cooperators they should survive, take over the population and cooperation should be inherently unstable. Kin selection theory has been invoked to help explain this dilemma^{32,33}. Put simply, by helping a relative reproduce, an individual indirectly passes its genes into the next generation. Kin selection has been proposed to be important in microbial social behaviors such as QS because of clonal reproduction and relatively local interactions^{26,34}. Recently the importance of kin selection in maintaining cooperative behaviors in single and multicellular organisms has been theoretically challenged, and microbial systems provide powerful experimental platforms to help resolve this debate³⁵⁻³⁷. The concept of kin selection is impacted by spatial structure, which can maintain cooperation and public goods because it keeps cooperators and relatives close together. Surface associated microbial communities (termed biofilms, described in detail later) are a good example of spatially structured populations. The biofilm lifestyle helps restrict the invasion of cheats and affects population dynamics^{28,38}. In addition, recent studies of QS have revealed molecular mechanisms for stabilizing cooperator populations. We have learned that metabolic prudence can constrain cheaters. Here an expensive public good is co-regulated by QS and nutrient availability such that the public good is produced when two conditions are met; there is a quorum and an ample nutrient supply such that the cost of public goods production is not critical³⁹. We have also learned that there are metabolic constraints on cheating where QS co-regulates private goods with public goods. Experimentally, transcriptomic studies revealed that although *P. aeruginosa* QS controls production of a battery of extracellular products, which may be considered public goods, it also controls some cell-associated products, one of which is a cellular enzyme required for growth on adenosine. As discussed above, when *P. aeruginosa* is grown on milk protein QS is required for expression of genes for extracellular proteases, and there is a fitness advantage for QS mutants. When *P. aeruginosa* is grown on milk protein plus adenosine cheats are unable to utilize the adenosine and their fitness advantage is nullified; a penalty has been placed on cheating⁴⁰. Finally, we are beginning to understand policing, defined as an ability of bacterial cooperators or a host organism (host sanctioning), to hinder the fitness of cheats⁴¹⁻⁴³. A recent report describes how QS regulation of pairs of genes coding for toxins and toxin immunity can serve as a policing mechanism. QS competent individuals deliver toxins to other individuals. QS competent cells are immune but QS mutants are not.

Although we now have a wealth of mechanistic data showing how QS systems function at the molecular level, the true biological function of many QS systems remains a research area of great opportunity. Evolution and ecological approaches can help address this knowledge gap. For example, are the chemically diverse QS molecules described in the literature always acting as signals? In an evolutionary context, a true signal evolved because it alters the behavior of a receiver and the receiver's response must have co-evolved. This is distinct from a 'cue' where the production of a substance has not evolved because of its effect on a recipient. If the production of a substance forces a costly response from a receiver we can differentiate this from signaling and term it coercion or chemical manipulation⁴⁴⁻⁴⁶. The current literature sometimes conflates signaling, cueing and coercion, and whether bacteria are interacting via a signal, a cue or coercion can lead to different biological outcomes. This

is becoming increasingly important as we begin to study microbiota interspecies interactions and as we seek to develop anti-QS therapeutics. It has been exciting for us to see these concepts introduced into the field of microbiology and to see the power of microbiology and microbial genetics brought to bear on questions about the mechanisms and consequences of communication and social interactions in ways that are immensely more difficult with higher organisms. Although there are certainly limitations in studies of social behavior and social evolution in bacteria there are important advantages. One can execute an experiment with hundreds of millions of individuals on the bench top. Experiments with ten generations of offspring can be done in a day. Correlating genes with social activity is routine. In this article, we focus a considerable amount of discussion on the pathogenic bacterium *Pseudomonas aeruginosa*. We do so in part because it is a particularly well-studied model in the QS field, and it is one on which the laboratories of all three co-authors have worked. There are mutant libraries of this bacterium. As a first analysis of gene-social function relationships one needs only to order a mutant and study it in the context of sociality.

How and why the distinction between signals, cues and coercion is important is exemplified by the AI-2 molecule described earlier (Fig. 1B). AI-2 is a furanosyl borate diester produced by *V. harveyi*⁴⁴. The identification of the *luxS* gene, which is required for AI-2 production⁴⁷, sparked a huge interest into AI-2 because this gene is found in both Gram-positive and Gram-negative bacteria¹⁵. This led to the hypothesis that AI-2 allows widespread communication between bacterial genera, a type of ‘bacterial Esperanto’⁴⁸. However, evolution and signaling theory question whether AI-2 can be defined as a true interspecies signal. For this to be the case AI-2 must (1) diffuse from the producing cell; (2) interact with a receiver cell; (3) elicit a response from the receiver cell that has co-evolved with signal production by the producer; and (4) benefit both producer and receiver. Points 1 and 2 are met with respect to AI-2, but points 3 and 4 are often not met between two or more species. Despite AI-2 being produced by many genera there are few instances, including *V. harveyi* luminescence⁴⁷ and the Lsr ABC transporter in *Salmonella typhimurium*^{49,50}, linking it with direct activation of specific genes. While a number of studies have reported that AI-2 ‘signaling’ impacts specific bacterial phenotypes, many of these studies have relied on the use of *luxS* mutant strains. As LuxS is involved in recycling of *S*-adenosyl-L-methionine, of which AI-2 is a non-toxic metabolic byproduct, *luxS* mutant phenotypes may simply be due to metabolic perturbations^{51,52}. In these cases, AI-2 cannot be considered a signal at either an intra or interspecies level. By understanding that in some cases AI-2 might serve as a signal where in other cases it is a cue, or even a waste product, studies of AI-2 should lead to evolutionary perspectives about how metabolic waste products can evolve to become signals, and about the evolution of chemical communication itself.

A next step: QS in natural habitats

The bulk of studies driving our current understanding of the molecular biology and evolution of QS utilize well-mixed laboratory cultures and growth environments not intended to mimic the natural environment. These *in vitro* systems provide reproducible conditions for biochemical and evolution studies, as well as the ability to grow large culture volumes often necessary for signal purification and identification. These systems have also provided a

wealth of solid fundamental knowledge on which we can build to study the role QS plays in modulating the composition and function of natural microbial communities.

Working in natural microbial habitats is challenging and requires QS scientists to embrace the complexity of these environments while leveraging state of the art multi-disciplinary approaches. One elegant system that has been developed to begin to study QS within a natural bacterial community in an animal host is the mutualistic symbiosis between the squid *Euprymna scolopes* and its light organ symbiont *V. fischeri*. The squid, which inhabits coastal waters of the Hawaiian Islands is born with a sterile light organ. The light organ is colonized specifically by *V. fischeri*, which occurs in low abundance in the surrounding seawater. *V. fischeri* uses its QS system to activate genes for luminescence in the high-density light organ environment. The mutualism is simple in that there are but two partners, and it is amenable to laboratory manipulation. This model system has provided insight not only about the role of QS in an animal host-bacterial interaction, but also about how a microbiome, albeit a simple one, can influence host development (for recent reviews see^{53,54}). Furthermore, juvenile squid are smaller than a pencil eraser and translucent, and bacteria tagged with GFP can be easily visualized in whole light organs. This has provided a means to study a difficult question discussed later in this review; can aggregates of bacteria employ QS signals to communicate with other aggregates, and if so is there a discrete distance over which this kind of communication can occur in a given condition (a calling distance)?

An ultimate goal is to integrate our comprehensive understanding of QS derived from elegant laboratory culture studies, with ecological principles to illuminate the role of bacterial communication in natural habitats. Researchers are now beginning to study more habitats with more complex microbiota than the squid symbiosis. Here, we focus on three areas of emerging interest that have important and relatively unknown functions in natural ecosystems: (1) orphan LuxR homologs; (2) the link between QS and microbial biogeography, and (3) quorum quenching.

Orphan or solo LuxR homologs and interspecies interactions—LuxR-type transcription factors consist of two domains, an N-terminal signal-binding domain and a C-terminal DNA-binding domain, and simple homology searches can be used to identify LuxR homologs in genomic sequences. Just such a search of the *Salmonella typhimurium* genome revealed the first example of what we now call orphan⁵⁵ or solo⁵⁶ LuxR homologs (Fig. 3A). The genome of *S. typhimurium* possesses a *luxR* homolog called *sdiA* but it does not possess a *luxI*-type gene, and *S. typhimurium* does not produce AHLs^{55,57,58}. In fact, many Gram-negative bacteria possess *luxR*-type genes and do not possess *luxI*-type genes, and many more Gram-negative bacteria possess greater numbers of *luxR*-type genes than *luxI*-type genes. In the case of *S. typhimurium*, we have learned that SdiA responds to AHLs produced by other bacteria, leading to activation of specific genes⁵⁸. There is also an *sdiA* gene in *E. coli*. The *E. coli* SdiA has been reported to respond to AHLs and mammalian host-produced small molecules^{59–61}. These latter studies provided the first hints that some orphan LuxR homologs might be involved in sensing the host environment, directly, rather than serving as QS signal receptors (Fig. 3A).

Some orphan LuxR proteins, such as QscR in the opportunistic pathogen *Pseudomonas aeruginosa*, can respond to self-produced AHLs^{62–64}, while others respond to non-AHL, self-produced signals. Two examples of the latter case involve members of the genus *Photorhabdus*. One species uses an orphan LuxR protein to detect self-produced α -pyrones⁶⁵ and the other detects self-produced dialkylresorcinols and cyclohexanediones (Fig. 1B)⁶⁶. Finally, there is a group of orphan LuxR homologs in some plant-associated bacteria that activate transcription of specific genes in response to small molecule(s) produced by the plant^{67–69}. The identity of these small molecules remains elusive and they may in fact be produced by the plant microbiota. The orphan LuxR homologs and what we now know about them bring this research area to an interface with advances in microbiome research, and studies of this group of LuxR homologs represent a rapidly emerging area.

Advances in mammalian gut microbiome research are beginning to reveal how QS can influence microbiome species composition, how QS research might lead to ways to control infectious diseases like cholera, and how the host itself may have evolved mechanisms to affect bacterial QS to shape its microbiome. For example, the AI-2 molecule produced by many bacterial species, and discussed earlier, was recently shown to promote Firmicutes over Bacteroidetes gut colonization⁷⁰ and production of AI-2 by a gut commensal bacterium can limit *Vibrio cholerae* infections⁷¹. Although the interactions of bacteria in the mammalian gut are much more complex than the two-partner squid symbiosis, it is becoming clear that the involvement of QS in these microbiomes can provide an opportunity to intervene in gut dysbiosis.

QS, biofilms and microbial biogeography—Biofilms are defined as high-density bacterial clusters frequently attached to surfaces and encased in an extracellular polymeric matrix⁷². Biofilm cells have several unique properties compared to their planktonic (free-living) counterparts, notably an enhanced tolerance to antimicrobials. Throughout the 1980s and 1990s, the prominence of biofilms in nature stimulated development of experimental laboratory systems. Several groups leveraged these systems for studying QS in biofilm communities. One common laboratory biofilm system is the flow-cell⁷³, in which biofilms growing on glass coverslips covering small channels are imaged using a confocal scanning laser microscope. A nutrient medium is continuously pumped through the channels to feed the biofilms. Using flow-cell systems, several groups showed that QS can impact biofilm construction as well as the ability of the biofilm to tolerate antimicrobial treatment⁷⁴. In some cases, QS is critical for building a biofilm, whereas in other cases it is important for biofilm disassembly. The link between QS and biofilm formation led to a flurry of studies to assess how microbial social behaviors impact this important mode of growth, but it was quickly discovered that this link was dependent on environmental conditions⁷⁵. Not surprisingly, there is interplay between environmental cues and intercellular communication. This leads to the question: Is the link between QS and biofilms specific to flow-cell laboratory conditions or is it relevant in natural ecosystems, and if so, which ecosystems?

We have learned that 3D biofilm architecture in flow-cells often does not mimic biofilm structure in natural ecosystems^{76,77}. Indeed, some naturally occurring microbial biofilms are composed of high-density, micron-scale aggregates containing hundreds to thousands of cells. These aggregates exhibit remarkable micron-scale spatial organization, and this

biogeography is critical for fitness of the microbial community^{78,79}. The fact that natural aggregates share traits with laboratory biofilms, including enhanced antimicrobial tolerance, led to the hypothesis that QS is involved in aggregate formation. This hypothesis is supported by studies of several bacteria including *Pantoea ananatis*, *Rhodobacter sphaeroides*, *Burkholderia thailandensis*, and *E. coli*. In *P. ananatis* and *R. sphaeroides*, QS inhibits formation of large aggregates via unknown mechanism(s), while aggregate formation is promoted by QS in *Burkholderia thailandensis* and *E. coli*^{80,81}. In the case of *E. coli*, aggregate formation is promoted via active movement of individual cells towards aggregates excreting AI-2⁸¹.

Although QS appears to play some role in aggregate formation, is it also important for the precise spatial organization of aggregates, and how does aggregate formation influence QS? To answer these questions, it is not only necessary to understand the relationship between aggregate size and QS-controlled behaviors but also the effective ‘calling distance’ of signals produced by an aggregate. Elucidating the number of cells required to reach a quorum has been actively pursued over the past 10 years. As predicted by the QS hypothesis, confining single bacterial cells in femto- to picoliter aqueous volumes has provided evidence that single cells can QS, although it is not clear whether there are fitness benefits to doing so^{82,83} (for reference the volume of an *E. coli* cell is on the order of about a half to one or two femtoliters). However, these closed systems do not allow exchange of solutes outside the confinement volume, which ultimately results in lack of robust bacterial growth. As microfluidics and laser printing technologies have continued to develop, it has become possible to address these sorts of questions by confining small numbers of bacteria in diffusive picoliter-scale hydrogel traps. One such study in which *V. harveyi* was trapped showed that aggregates with diameters of about 25 microns demonstrated robust QS while those ≤ 10 microns showed little QS-dependent gene expression⁸⁴. Similar results were observed for *P. aeruginosa*, which had been confined in micro-3D printed bacterial ‘lobster traps’. As few as 500 *P. aeruginosa* cells were shown to produce the QS-controlled exoproduct pyocyanin when confined within 8 picoliter traps, indicating that aggregates of this size are capable of initiating social behaviors in an open system⁸⁵.

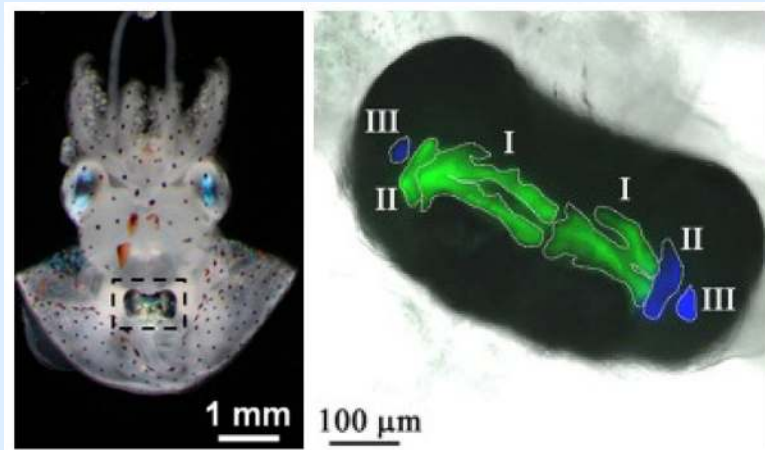
A next step in understanding bacterial communication revolves around whether aggregates can communicate with each other: What is the ‘calling distance’ of QS signals, how far can two aggregates be from each other and still interact? Several theoretical studies have focused on this question, and the general consensus is that communication at distances greater than 10–100 microns is unlikely^{86–88}. One empirical study provided a cursory set of experiments aimed at assessing communication between spatially organized aggregates⁸⁵. This study employed state-of-the-art laser-printing technology to trap *P. aeruginosa* in aggregates separated by 8 microns. The ability of one aggregate to communicate with another via AHL QS was assessed for aggregates of different sizes. Signaling across this 8-micron distance required signal-producing aggregates to contain at least 2,000 cells. Although *P. aeruginosa* aggregates greater than 2,000 cells have been observed in natural ecosystems including chronic infections^{76,89}, most aggregates in these communities are generally smaller and are often spaced further than 8 microns apart (Fig. 3B). These data suggest that in some environments, *P. aeruginosa* QS may primarily function as an intra-aggregate communication system. The squid-*V. fischeri* symbiosis provides a unique opportunity to

address questions of inter-aggregate signaling in a natural host-associate habitat (see Box 2). We believe that there is much yet to learn about how micron-scale biogeography influences how bacteria interact. This will be critical as we begin to manipulate microbial communities such as the human gut microbiome by influencing QS either chemically or using probiotic approaches.

Box 2

Biogeography of *V. fischeri* in a squid light organ

As described in the text the squid-*V. fischeri* light organ mutualism has proven to be a useful two-partner model for fundamental studies of quorum sensing in a real-world host-microbe setting. It is also becoming an important model for biogeography studies concerning the ability of spatially segregated aggregates to communicate with one another. The light organ consists of two lobes, and each lobe possesses three crypts. Descending into a lobe the crypts become progressively smaller. If the experimenter presents a new born squid with a mixture of *V. fischeri* mutants or strains in the surrounding seawater different crypts can be colonized with different mutants or strains. We know this because *V. fischeri* can be tagged with genes coding for different fluorescent proteins and whole light organs can be imaged by fluorescence microscopy (see image below). AHL QS signals can diffuse through the crypt barriers. Can an AHL signal produced by a wildtype *V. fischeri* in one crypt activate the luminescence genes in an adjacent crypt? Can wildtype in a small crypt activate bacteria in a larger crypt or perhaps maybe signaling is unidirectional depending on crypt size? Can a signal-producing luminescence mutant activate luminescence by a signal-negative mutant in an adjacent crypt, and will the luminescent strain compensate for the luminescence mutant by producing more light itself? The answers to these questions will be fascinating, and coupled with laboratory experiments on physically separated aggregates of bacteria will provide critical information as we begin to understand the social interactions in more complex ecosystems like the human gut.



Box 2 Images.

Left, Juvenile squid with infected light organ (boxed). The mantle has been dissected to expose the light organ. Right, Fluorescence microscopy of light organ crypts colonized

with a mix of YFP-labeled *V. fischeri* FQ-A001 and CFP labeled strain ES114. The three crypts in each lobe are outlined and labeled I, II and II. Images provided by T. Miyashiro Penn State University. Details of this type of experiment have been published previously¹¹².

Quorum quenching in natural ecosystems and as a therapeutic modality—As it is clear that many natural microbial communities are polymicrobial and spatially structured, it is important to consider how ecological interactions between species shape the evolution of signaling. Important considerations include abiotic and biotic factors that interfere with QS through degradation of signals (Fig. 3C), a process termed quorum quenching (QQ)⁹⁰. Signal degradation can result from the chemical characteristics of an environment such as pH, or due to the action of enzymes produced by microbes or animals. In the latter case, two main types of AHL-degrading enzymes, lactonases and acylases, have been described. Lactonases hydrolyse the HSL ring of an AHL to produce corresponding acyl homoserines⁹⁰, whereas acylases cleave the AHL amide bond generating the corresponding fatty acid and homoserine lactone⁹¹. Determining the impact of QQ on natural microbial communities, given the complexities of spatial structure and signal calling distance, remains a key challenge for the future. For example, are QQ enzymes produced for competition, cooperation or for the private benefit of producing cells? It is notable that humans also possess lactonases (the paraoxonase (PON) family of enzymes), which are better known for their ability to hydrolyze organophosphate toxins and low density lipoproteins^{92–95}. PON lactonase activity is considered to be the ancestral activity^{92,94}, and *Drosophila* PONs appear to serve a host-defense function⁹⁶. It is possible that such enzymes provide the host a means of manipulating the microbiome through modulation of microbial social interactions.

Regardless of the true functions of QQ enzymes, QQ presents an attractive and progressive route for treating the rising number of antimicrobial resistant infections. A recent report commissioned by the Wellcome Trust estimated that by 2050, antimicrobial resistance (AMR) could cause 10 million additional deaths annually, and a cumulative loss to the worlds GDP of \$100 trillion⁹⁷. QQ enzymes and other QS-blocking approaches do not kill pathogens but instead block virulence factor production, and such anti-virulence agents have been proposed to impart less selective pressures that lead to the development of resistant mutants⁹⁸ (see Box 1). The basis for QS inhibition as a therapeutic approach dates back to the 1990s, when a brominated furanone produced by the Australian macro-alga *Delisea pulchra* was shown to antagonize AHL-controlled phenotypes in a number of bacterial species⁹⁹. Importantly, halogenated furanones have been shown to be effective *in vivo*, resulting in clearance of *P. aeruginosa* from the lungs of infected mice¹⁰⁰. Subsequent studies identified a number of small molecule inhibitors of AHL QS, many of which have potent activity against several bacterial pathogens laboratory culture^{99–104}.

What diseases might be treated by QS inhibition? Obviously, we would like to be able to treat infections that are not resolved by current therapies. The chronic *P. aeruginosa* lung infections, which plague people with the genetic disease cystic fibrosis (CF), have been an

inviting target, and targeting chronic *P. aeruginosa* infections presents a clear example of why fundamental research and therapeutic development are co-dependent and must proceed hand-in-hand. These chronic infections are not resolved by antibiotics, and the chronic microbial communities residing within the CF lung are comprised of bacterial aggregates⁸⁹. Because QS activates a battery of *P. aeruginosa* virulence factors, and *P. aeruginosa* QS mutants have reduced virulence in animal models, LasR, the master QS signal receptor became a focus of small-molecule inhibitor screens^{103,105,106}. Subsequent ecological studies showed that many patients harbor *P. aeruginosa* LasR mutants^{107,108}. These ecological studies were of course discouraging, but further investigation showed most LasR mutant CF isolates have co-opted a second QS system, the RhlR system, to replace LasR in ways, which remain unclear¹⁰⁹. Perhaps a RhlR inhibitor or an inhibitor that targets both RhlR and LasR might be an appropriate CF therapeutic, and one has recently been reported⁹⁴. The lesson is that we need continued basic research about QS in natural human habitats to know how, when or what to target with a QS inhibitor.

In conclusion, there has been an explosion of activity on bacterial QS and the field continues to expand rapidly. One area ripe for advancement involves complex adaptive microbial communities. QS likely controls behaviors critical for development and success of these communities in diverse environments like the human gut microbiome and chronic infections of humans. Elucidating the roles and functions of QS in natural ecosystems requires a continued willingness to embrace the complexity of these microbial communities and incorporate systems level ecological principles. We will continue to see incorporation of the fundamental biology of bacterial social dynamics into thinking about how to interfere with certain infectious diseases, and there will be a continued use of bacteria to understand the biology of communication and sociality. We have come to understand that there are many different small molecule-dependent interactions between microbes and between microbes and their hosts. There are certainly more to be discovered and there is an opportunity to sort out fundamental differences between these diverse systems. Understanding these issues will be critical as we move towards translating basic studies of QS to meet future needs including functional studies of the human microbiome.

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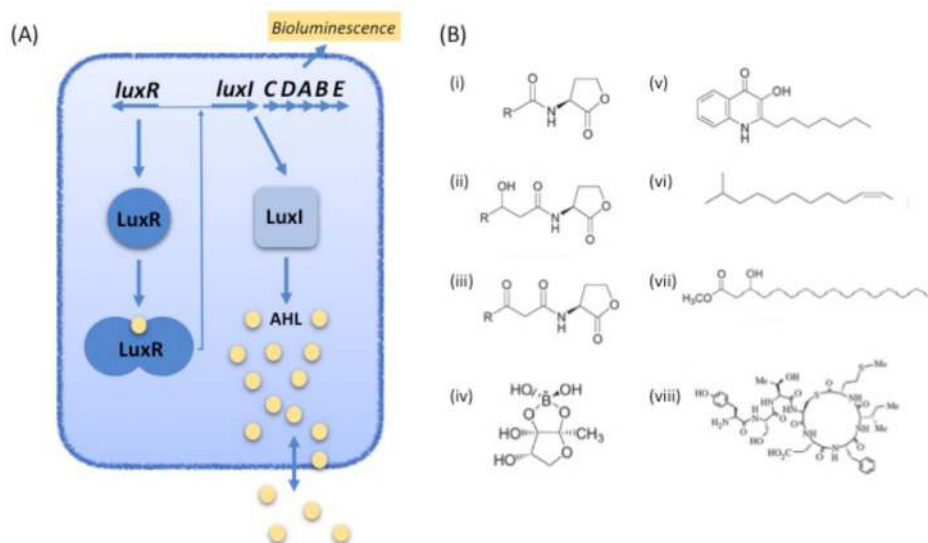


Fig. 1. Canonical QS and the chemical diversity of signals

(A) Quorum sensing in *Vibrio fischeri*. LuxI produces 3O-C6-HSL (AHL, yellow spheres), which specifically interacts with the LuxR transcriptional regulator when it reaches concentrations in the nM range. This leads to expression of the *luxICDABE* operon and bioluminescence. (B) Examples of quorum sensing signals from Gram-negative and Gram-positive bacteria. (i) AHL, *N*-acyl homoserine lactone; (ii) 3-Hydroxy-AHL, *N*-(3-hydroxyacyl)homoserine lactone; (iii) 3-oxo-AHL, *N*-(3-oxoacyl)-*L*-homoserine lactone. R can be a fatty acyl group of 4–18 carbons with or without one unsaturated carbon-carbon bond, the terminal carbon can be branched and some R groups are aromatic acids (*p*-coumaric acid or cinnamic acid); (iv) The *V. harveyi* AI-2, autoinducer-2, furanosyl borate ester form; (v) PQS, *Pseudomonas* quinolone signal, 2-heptyl-3-hydroxy-4(1H)-quinolone; (vi) DSF, diffusible factor, methyl dodecenoic acid; (vii) PAME, hydroxyl-palmitic acid methyl ester; (viii) Autoinducing peptide 1 (AIP-1) from *Staphylococcus aureus*.

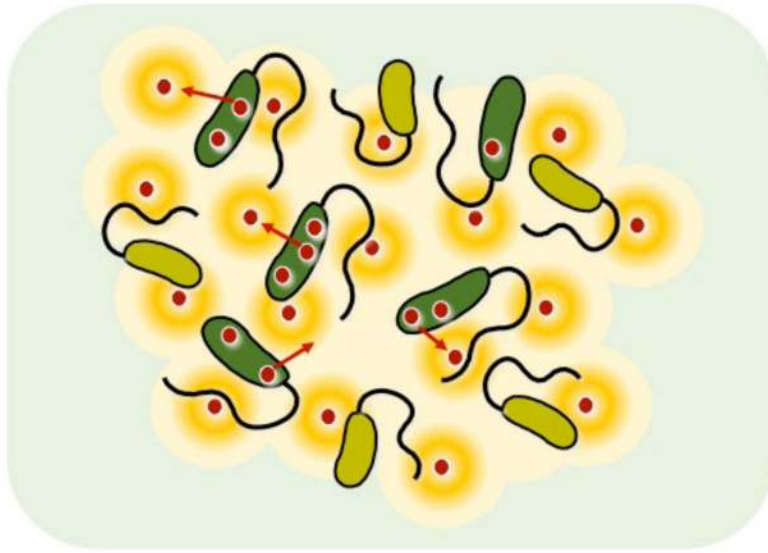


Fig. 2. Social cheating in QS populations

Bacterial cells act as cooperators (green) when secreting QS-dependent public goods (e.g. protease, red spheres) into the surrounding environment and this imposes a fitness cost on individual cells. Cheater cells (yellow) do not secrete these enzymes and pay no fitness costs. Cheats can benefit from the action of public goods and therefore gain a fitness advantage in mixed populations with cooperators. Orange halos depict a nutrient source liberated by a public good (e.g. protease) from which all cells (cooperators and cheats) can benefit.

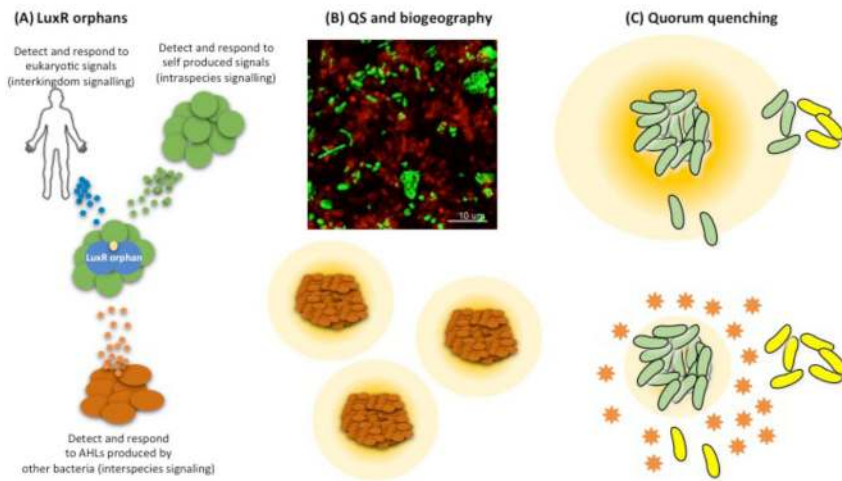


Fig. 3. Factors that impact QS in natural environments

(A) LuxR orphans (solos) can detect and respond to signals produced by eukaryotic hosts, self, or other microbial species. (B) Top: Aggregates of two *P. aeruginosa* strains (green, strain PA14 and red, strain PAO1) in synthetic cystic fibrosis sputum¹⁰¹. Bottom: Diagram of *P. aeruginosa* aggregates (3 clusters of red rods) spatially separated and socially isolated (yellow halos represent QS signals and QS-controlled exoproducts). (C) Top: Diagram of a high-density aggregate of QS positive cells (green) secreting a diffusable QS signal into the surrounding environment (orange halo). The signal can activate QS in nearby cells (green) but not in more distant cells (yellow). Bottom: quorum quenching (orange stars), either by the action of enzymes or due to environmental conditions, degrades the QS signal and limits the ability of an aggregate to induce QS in nearby cells.