Historically, conventional thinking in evolutionary biology has drawn a bright line separating multicellular from unicellular organisms. Only the cells of animals, plants, and fungi were thought capable of achieving the synchrony required to function as a collective. Bacteria, in contrast, were perceived as blind, deaf, and mute, with each cell single-mindedly focusing on its own metabolism and reproduction. However, recent discoveries in the field of bacterial cell-cell signaling have exposed the ubiquity of multicellular behavior in bacterial populations, dramatically altering our understanding of the microbial world.

**Introduction to Quorum Sensing**

The first complete descriptions of cell-cell signaling systems used by microbes to regulate gene expression were obtained from studies of the marine bacteria *Vibrio fischeri* and *Vibrio harveyi* over 30 years ago (1). The genes studied encode light-producing luciferase enzymes and are up-regulated in response to increases in population density (1). Underlying this unusual behavior is a pheromone, or autoinducer, from the family of acylhomoserine lactones (AHL) (2). Every *Vibrio* cell contains the gene *luxI*, whose protein product, known as a synthase, synthesizes AHL (2). As the cells divide, increasing their population density, the concentration of AHL also increases (2). An intracellular receptor, encoded by the gene *luxR*, binds AHL when the concentration of the pheromone reaches a specific threshold (2). The LuxR-AHL complex acts as a transcriptional activator, stimulating production of the luciferase enzymes and LuxI, which produces the original autoinducer (2). Further increases to the concentration of LuxI establish a positive feedback loop, leading to population-wide production of light (2).

In some respects, the mechanism of cell-cell signaling used by *Vibrio* cells to assess population density resembles a democratic system of government. Each cell casts a vote through its contribution of pheromones. When cells detect the presence of a sufficient concentration of pheromone, analogous to the recognition of a quorum, the group takes action. A nonscientist friend of Stephen Winans, a researcher at Cornell University studying the role of bacterial signaling in plant crown gall disease, serendipitously devised the term “quorum sensing” to describe multicellular bacterial behavior (3). As described by microbiologists, quorum sensing refers to the ability of bacteria to detect population density and coordinate corresponding shifts in gene expression patterns (4).

**Multicellular Bacterial Behaviors Regulated by Quorum Sensing**

Transcriptional regulatory proteins activated by binding to pheromones enhance gene expression in genes whose promoters contain a specific sequence element recognized by the protein-pheromone complex. While the synthases of pheromones and their associated receptors originated early on in bacterial evolutionary history — the LuxI/R system of the *vibrios* has homologues in evolutionarily distant human and plant pathogens — even closely related quorum sensing systems can be repurposed to control a variety of behaviors (5). Behaviors activated in density-dependent response pathways include bioluminescence, symbiotic plant root nodule formation by nitrogen fixers, bacterial mating, antibiotic production, biofilm formation, and extracellular DNA uptake in response to harsh conditions (6). In all of these cases, bacteria only benefit from engaging in these activities collectively at high cell densities.

*Vibrio fischeri*, for example, engages in a partnership with the Hawaiian Bobtail Squid by producing light when the bacteria grow in the nutrient-rich environment of the squid’s specialized light producing organs. This relationship provides direct benefits for the squid, which can escape the...
Vibrio fischeri has a symbiotic relationship with the Bobtail Squid. By using the light produced by their complement of V. fischeri, the squid can eliminate telltale shadows on the seabed. Light production only confers a selective advantage for bacteria when they are growing within the confines of the squid. Free-floating populations of V. fischeri do not express high levels of luciferase. Using the LuxI/R quorum sensing system, the bacteria are able to avoid the individual fitness cost of luciferase synthesis in the open ocean.

While many bacterial species, including V. fischeri, do no harm to the hosts they colonize, quorum sensing also aids bacterial pathogens. Highly developed immune systems, like those of humans, are adept at preventing infectious disease; however, bacteria are able to avoid a host immune response by suppressing expression of virulence factors until enough cells are present to overrun the host (7). Interestingly, not all pathogens conform to this model of virulence regulation by quorum sensing. In Vibrio cholerae, the etiological agent of cholera, a two-pheromone quorum-sensing network arranged in parallel represses virulence factor production at high population densities, but permits their expression at low densities (2). More background on the parthenogenesis of cholera reveals the evolutionary rationale for this idiosyncrasy, as it is the diarrhea resulting from cholera that allows V. cholerae to spread (2). Concurrent inhibition of biofilm formation permits bacteria to detach from their intestinal hideout and “go with the flow” (2).

Biofilm formation, involving the creation of multicellular aggregates, is an important intermediate step in the progression of bacterial infection. Although significant variation exists in the structure and composition of biofilms, most bacterial species create biofilms through the secretion of exopolysaccharides, the primary constituent of the extracellular matrix that binds cells into a biofilm (8). Bacteria in biofilms are more resistant to antibiotics and the host immune system, properties useful for maintaining chronic infections (9). It is estimated that biofilms participate in 65 percent of human bacterial infections, making biofilm formation and maintenance the focus of extensive research (8). Perhaps not unexpectedly, quorum-sensing signals have been shown to make important contributions to biofilm development in many species of microorganisms (8).

Disrupting Quorum Sensing Systems

By employing intercellular signaling, bacteria enjoy a seemingly limitless ability to adapt to the vagaries of their environment. Our body’s defenses are all too often unable to adequately respond to the challenges posed by a cooperative group of bacteria producing virulence factors or encasing themselves in protective biofilms. Fortunately, this model of host-bacterial interaction, in which bacteria always have the upper hand, is only partially correct. While fighting infections by Pseudomonas aeruginosa, a process dependent on quorum sensing, the human body is able to use its paraoxonase family of organophosphate-hydrolyzing enzymes to degrade the acyl homoserine lactone pheromone produced by the bacteria (6). Inhibition of quorum sensing, or “quorum quenching,” has also been observed in barley and fungi. This implies that quorum quenching is an evolutionarily successful strategy for combating bacterial infection (10).

In an era dominated by multi-drug resistant bacteria, alternatives to antibiotics active on a broad class of microorganisms are in high demand. It is a sobering thought that Penicillium fungi, from which the “miracle” drug penicillin was derived, produce small molecules that inhibit quorum sensing (6). These molecules mimic the pheromones of specific bacterial species, competitively binding to receptors of the true pheromone to prohibit activation of the quorum-sensing pathway (6). Apparently, even the organisms responsible for sparking the mass production of antibiotics cannot solely rely on that class of biomolecules for protection. Biotechnology may yet restore Penicillium fungi to the forefront of anti-microbial warfare due to recent advances in methods of disrupting quorum sensing.

The quorum sensing systems of gram-negative bacteria use acyl homoserine lactones as primary pheromones. Despite the potential for cross-species signaling, most pheromone-receptor systems are used for intraspecies communication, specific to a single bacterial species or strain (11). Exceptions do exist, however. For example, the synthase of AI-2, a pheromone struc-
for intercellular signaling. From a bioengineering perspective, quorum-sensing bacteria create tantalizing possibilities for gene circuit design, in which well-defined chemical inputs are linked to desired outputs (6).

One astonishing multicellular machine has already been developed: a micro-scale clock driven by the oscillating fluorescence output of recombinant bacteria (15). The gene circuit driving this behavior contains components from the LuxI/R pheromone-receptor pair of the *Vibrio*, but links their activation to the expression of Green Fluorescent Protein and an enzyme that quenches the LuxI/R pheromone signal (15). Coupling the transcriptional activator to its own repressor leads to the establishment of tunable, periodic fluorescence (15). Removal of excess extracellular AHL via a microfluidics system is required for the maintenance of oscillation, which demonstrates, as in the *V. fischeri* — squid symbiosis, the importance of environment to the long-term stability of quorum sensing behaviors (15).

**Conclusions**

From bacterial biofilms to networks of neurons, life depends on cellular communication as the basis of its continued survival. Although the methods of intercellular communication employed by plants, animals, and fungi are far more complex than those of bacteria, the existence of quorum-sensing systems indicates that precursors of multicellularity penetrate further into the tree of life than we previously expected. Moreover, the regulatory networks associated with quorum sensing are hardly archaic. Humans consist of roughly one trillion cells, far outnumbered by the ten trillion bacterial cells estimated to reside on or around our body (16). Quorum sensing systems mediate interactions between our body and its associated microbes that enrich our lives tremendously by aiding us in digestion, protection against environmental hazards, and vitamin synthesis (16). Conversely, quorum sensing is used to maximize harm to our body by invasive pathogens, often overshadowing the positive contributions of our microbiome.

The challenge of fully elucidating quorum sensing will continue to stimulate research into bacterial communication in the near future. It is likely that bacteria have been communicating using pheromones for billions of years. For the first time in history, when bacteria talk, the world listens.

**References**