Quorum Sensing and Social Interactions during Infection

Social evolution is an active area of research in microbiology, opening new approaches to understanding environmental adaptations, including virulence

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Bacteria engage in diverse communal behaviors, such as forming biofilms, exchanging DNA, coordinating the production and release of enzymes and toxins, and moving in swarms. Scientists working in the branch of evolutionary biology called social evolution recently began to ask why individual cells interact in these ways.

The ideas underlying social evolutionary theory derive from studies of helping behavior, group formation, and reproductive cooperation in birds, mammals, and social insects such as bees and ants. A major historic focus of this work was to explain how these behaviors arose and what forces maintain them. Explaining why cooperation exists is problematic because many such behaviors appear to be costly. For example, the immediate effect of forgoing reproduction to help take care of another individual’s offspring, joining in a fight to support another individual, or sharing food reduces the fitness of the actor performing the behavior but increases the fitness of the recipient(s). Charles Darwin alluded to this problem, noting how insect drones give up reproducing for the benefit of their hive queen when it would seem to be more directly beneficial for them to breed themselves.

However, during the 1960s, William Hamilton of Imperial College in London, United Kingdom (UK), showed that individually costly behaviors could be deemed successful in evolutionary terms if they benefitted close kin. He termed this “inclusive fitness,” distinguishing it from fitness benefits that are purely “direct,” which only accrue to the actor. Inclusive fitness includes benefits that are direct but also ones which are “indirect,” which accrue to others who share the same genetic predisposition to altruism. Inclusive fitness is more commonly referred to as “kin selection” and forms the basis of the study of social evolution.

Social evolution has become an active area of research in microbiology for a number of reasons. First, microbes display many apparently social or cooperative behaviors, and many of these behaviors seem to be crucial for those microbes to survive in their ecological niches or to become successful pathogens. Biofilms are a prime example, as they allow microorganisms to colonize surfaces despite abrasion or flow, and they help to protect the microorganisms against desiccation, antimicrobial compounds, and host immune systems. Social evolution research also complements mechanistic studies—for example, by presenting problems for which there must be mechanistic solutions. Further, environmental conditions can be experimentally manipulated to test evolutionary theories that apply to microorganisms. Finally, rapid generation times allow for evolution experiments involving microbes to be run over many generations, with a high level of experimental control.

SUMMARY

- Individual microbial cells may interact in a variety of ways, including through quorum sensing (QS), coordinating production of nutrient-scavenging molecules, and forming multicellular biofilms.
- Understanding whether a behavior is social can help us understand how bacterial populations interact within infected hosts, explaining some kinds of clinical observations as well as how virulence evolves.
- Despite interest in this issue, experimental tests of whether bacterial behaviors such as QS are truly social are scarce.
- From both mechanistic and evolutionary standpoints, QS is a social behavior.
- Mutants that are defective in QS can be isolated from sites of infection, despite QS being important for virulence.
What Is Social Behavior among Microbes?

A social behavior is one that affects individuals other than, or in addition to, the individual performing that behavior. Behaviors are classified into four main types based on whether they confer a cost or a benefit to the actor and the recipient: these are mutual benefits, altruism (both forms of cooperation), selfishness, and spite (Fig. 1A), according to Stuart West, now at the University of Oxford in the UK, and his collaborators.

A basic and well-described form of cooperation in bacteria is the secretion of proteins and lower-molecular-weight compounds that enable cells to scavenge essential nutrients and resources from the environment. Such “public goods” include proteolytic enzymes, toxins, and iron-scavenging siderophores, which can be important virulence factors when the environment is an infected host. Producing these molecules is costly for individual cells. However, the benefits of their activities accrue both to producer and neighboring cells, regardless of whether the neighbors themselves make the costly exoproducts.

Thus, cells that cease to make the public good can avoid the production cost, but still benefit from the nutrients those compounds release through their interaction with the environment. They gain an increase in benefit in mixed populations with cooperators.

FIGURE 1

(A) Social interactions. Social interactions are divided into 4 groups based on whether they benefit or are costly to the initiator and/or recipient of the behaviour. (B) Social cheating. Bacterial cells act as cooperators when secreting compounds (e.g. enzymes) and this imposes a fitness cost. Cheater cells do not secrete these compounds but can benefit from the nutrients those compounds release through their interaction with the environment. They gain an increase in benefit in mixed populations with cooperators.

Cooperative cells produce public goods. Cheater cells do not

All cells, whether they contributed or not, benefit from the availability of public goods.
terminating whether cooperators or cheats win out over evolutionary time. When cooperative acts are targeted towards relatives that also carry cooperative genotypes, cheats cannot easily gain a foothold. The spatial structuring of populations that enables such cooperative acts typically depends on limited dispersal of individuals or aggregates forming from clone-mates. Alternatively, when bacteria live in a group and the success of the individual is tied to the success of the local population—for example, because cells from more productive patches are more likely to colonize new patches—then the evolutionary interests of the individual and the group are aligned and groups containing cheats may die out.

Through the use of powerful techniques such as genomic sequencing, we are learning a huge amount about the mechanisms underlying bacterial social behaviors. But while we know a lot about how gene expression and protein translation are controlled, we still do not know the answers to basic questions about how these behaviors evolved, how they are maintained in natural populations, and whether they are social in nature. These questions matter, not least because in the case of pathogenic bacteria, they influence virulence and antimicrobial resistance.

**Testing Whether a Microbial Behavior Is Social**

To determine whether a microbial behavior is social, a number of key experimental steps need to be followed. First, defined mutants resulting in the abolition or reduction of the behavior need to be identified to demonstrate reduced fitness in an environment where the behavior provides a benefit to cells (Fig. 2). For example, in low iron conditions, siderophore mutants grow poorly compared to wild-type cells. Second, when mutants and wild-type cells are grown in mixed populations, mutant fitness should increase as they exploit wild-type cells that are producing useful molecules (Fig. 2). Finally, the fitness of a particular type of mutant should negatively correlate with its starting proportion in a mixed population. Put simply, as social cheats become more common, fewer wild-type producing cells will be present within the population to be exploited, resulting in decreased benefits to cheating.

West and his collaborators empirically demonstrated cooperative social behaviors in the form of siderophore production in *Pseudomonas aeruginosa*, and Joan Strassmann and David Queller at Washington University in St. Louis, Mo., revealed cooperative reproduction in slime
molds. When slime molds exhaust available nutrients, they produce a specialized fruiting body. Some cells differentiate into reproductive spores, while other cells form a stalk that holds the spore population aloft. The stalk increases the distance spores can disperse, maximizing their chances of finding a favorable habitat to germinate. Although stalk cells do not themselves reproduce, cooperative genotypes of slime mold differentiate into stalk and spore cells with equal probability. However, some cheating mutants preferentially become spores, increasing their fitness relative to cooperators.

The production of siderophores, small molecules that scavenge iron, by *P. aeruginosa* demonstrates cooperation in a more fundamental way. Where growth is restricted by iron availability, wild-type production of siderophores can be exploited by siderophore-null mutants, which act as social cheats. This neatly demonstrates that producing a costly public good can be classified as a social trait and implies that social behaviors may be common in bacteria.

**Quorum Sensing Provides Another Example of Social Behavior**

Our work focuses on bacterial quorum sensing (QS), a process whereby cells communicate with each other via diffusible signal molecules. These signals coordinate a wide range of behaviors at the population or group level. In a range of gram-negative and gram-positive bacterial species, QS signals regulate the production of extracellular “public goods,” including nutrient-scavenging molecules, toxins, immune suppressants, and surfactants that aid cellular motility.

Signal molecules are usually produced at a basal rate that does not induce these behaviors. However, when a population reaches a high density, the levels of the signal molecule also increase, leading to a positive feedback mechanism, which results in a considerable increase in signal and QS-controlled factors (Fig. 3). This coordinating function of QS and its link with public goods production indicate that it is a social behavior.

The fitness benefits of QS in *P. aeruginosa* are density dependent, and QS in both *P. aeruginosa* and *Staphylococcus aureus* is both costly and exploitable by cheats. Using a synthetic growth medium where QS is important for bacterial growth due to production of costly exoproteases, wild-type *P. aeruginosa* populations grow well, but populations of *lasR* mutants, which do not respond to QS signals, grow poorly. Crucially, in mixed culture, *lasR* mutants act as cheats: they have a fitness advantage because they exploit the exoproteinase production of wild-type cells, ac-

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**FIGURE 3**

The core dynamics of quorum sensing. (A) At low cell densities, a low level of signal is secreted and this is insufficient to activate a QS receptor protein. (B) as the number of bacteria in a space increases so does the level of signal. (C) At a given threshold (quorum) the signal can activate the receptor, which in turn causes a greater production of signal and receptor as well as up-regulating QS controlled genes, either directly or through secondary messengers.
Diggle: Plagues, Misdemeanors, and a Long-Term Interest in Bacteria

Steve Diggle traces his dual passion for microbiology and music to his primary school teacher Mrs. Liversage, who taught his class about the Great Plague of London of 1665 and, separately, showed him how to play a few chords on a guitar. “From that moment I developed an unhealthy obsession with plague and read lots of books on the subject. Learning about the plague when I was 10 years old was simply a revelation to me,” he says.

Diggle’s interest in bacteria continues, as does his guitar (bass) playing. At age 19, he and several of his friends formed a progressive rock band called Mr Meaner. “The name was supposed to be an amusing play on the word misdemeanor,” he says. “It was funny for about 10 minutes and then it became annoying, but we stuck with it.” After calling it a day in 1995, Mr Meaner recently revived to record what they should have done 20 years ago. “Being in a band teaches you skills that are important for science,” he says. “Perseverance… coping with rejection… collaborating on projects… being able to give and take criticism, keeping your feet on the ground, and not getting too bothered about things.”

Diggle is associate professor in the University of Nottingham Centre for Biomolecular Sciences, where he studies the social behavior of microbes—specifically, cooperation and communication, and their implications for microbial virulence. He also is interested in ways to treat infections and is collaborating with colleagues in the School of English to determine whether medieval and Anglo-Saxon recipes will work against organisms that are resistant to conventional antimicrobial agents.

Diggle, 44, was born and grew up in Stockport, an industrial town in northwest England, about seven miles from Manchester, which accounts for him being a Manchester United fan. His parents, now retired, were high school teachers, and his grandmother, who died this year at 100, was a particular inspiration to him. “She was just a remarkable lady who lived through a lot,” he says. “She was a missionary in Ethiopia in the late 1960s when it was a dangerous place to be. She and my granddad pretty much sold everything to go and help people, which I always found amazing.”

Diggle was not a high school standout. “I left with pretty much no qualifications,” he says. At 17, he began working in a small company that isolated a compound from rabbits that was used to test the clotting time of blood. “My job was to remove 200 brains from rabbit heads that we got every day from the local abattoir,” he says. “I got pretty fast at it.”

After 18 months, Diggle moved to work at Withington Hospital before moving to the Paterson Institute for Cancer Research, also in Manchester, he says. “However, I knew I needed to get a degree to move on.” Before attending Salford University full time in 1993, he did a “one day a week access course in science for two years,” he says. He graduated from Salford in 1997 at age 27. He earned his doctorate from the University of Nottingham in 2001, and then did postdoctoral research until 2006.

Diggle’s wife Fran teaches biology in an independent school, and their son, Angus, is 12. While the band still takes up most of his free time, he also likes to read. “I’m a bit of a Tolkien nerd, and I like the Game of Thrones novels and TV series,” he says.

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.

According to our findings and those of Martin Schuster at Oregon State University in Corvallis and his collaborators.

Similarly, when mice with burned skin or with chronic wounds are infected with *P. aeruginosa*, we found that *lasR* mutants act as cheats and can invade this bacterial population within days. In waxmoth larvae (waxworm) infections, we showed a similar pattern of sociality for *S. aureus* *agr* mutants. The mutants are less fit than their wild-type counterparts in monoculture infections but demonstrate social cheating when in mixed infection.

These results suggest that, during infection, QS cheats can exploit public goods and resources produced by a cooperating population to gain a fitness benefit. In addition, mixed infections of cooperating and cheating cells of *P. aeruginosa* and *S. aureus* are less virulent than are unmixed infections. Therefore, the spread of cheats in a population can significantly alter the outcome of an infection. Even within a single species, significant phenotypic diversity can evolve during infection, magnifying the importance of this phenomenon. Social evolution theory can help to explain how and why such diversity arises and its implications for virulence, infection, and antibiotic resistance.

Such results also help to explain why QS mutants arise in clinical infections even though such loss-of-function mutations might appear to be detrimental to fitness. Both *agr* and *lasR* mutants...
have been isolated from different types of infections, particularly chronic infections. The predominant types of mutation affect the ability of cells to respond to signals, rather than to make signal molecules. Theory suggests such "signal-blind" mutants make better cheats because they can no longer respond to signal and thus cannot cooperate with wild-type bacteria to make costly public goods.

However, although QS systems can be social both in vitro and in vivo, it is important to realize that the growth environment can alter the social nature of traits. For example, we recently showed that, in porcine lung tissues, QS may not provide any social benefits. In this system, lasR mutants do not cheat, they simply appear to grow better.

This result could mean that QS P. aeruginosa mutants in infected cystic fibrosis patients arise due to adaptation rather than through social cheating. Furthermore, QS in P. aeruginosa does more than control traits that can be considered public goods. Some enzymes controlled by QS are intracellular, and break down nutrients for growth within the cell, providing direct benefits for the producer cell. By regulating private goods, this metabolic component of the QS system may well restrain social cheating, according to Peter Greenberg of the University of Washington in Seattle and his collaborators. Therefore the growth environment can change the social dynamics of traits, highlighting the need for further testing which traits are social in different environments.

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