Selection favors incompatible signaling in bacteria

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A cooperative group can achieve more than the sum of its members. Evolution has taken advantage of this principle in most natural systems, from multicellular individuals to ant colonies. To do so, it has provided the members of cooperative groups with communication tools, which are critical for effective cooperation. For example, some ants form bridges with their bodies to help their nest-mates cross a gap (1, 2). But this admirable behavior only makes sense when many ants mass along the same route; a lone scout that stayed put across a gap instead of wandering off in search for food would do a disservice to the colony. Similarly, many bacteria cooperate in ways that only make sense in large groups, for example secreting a sticky goo to keep bacteria together forming a biofilm, or a slippery one to help movement (3). To prevent wasting resources on these public goods when bacterial density is too low to have an advantage from them, many species measure local bacterial density using a mechanism called quorum sensing, and produce the public good only when numbers are high enough to make it count (4). This function of quorum sensing seems straightforward, but one piece of information does not quite make sense: in natural populations, different individuals have different—and incompatible—quorum-sensing machineries (5). If the bacteria are trying to coordinate with their neighbors, why do they use a different signaling system? In PNAS, Pollak et al. demonstrate an elegant answer to this question: a rare mutant with incompatible quorum-sensing machinery initially exploits the wild-type, but is able to cooperate with its own kind when common in the population (6).

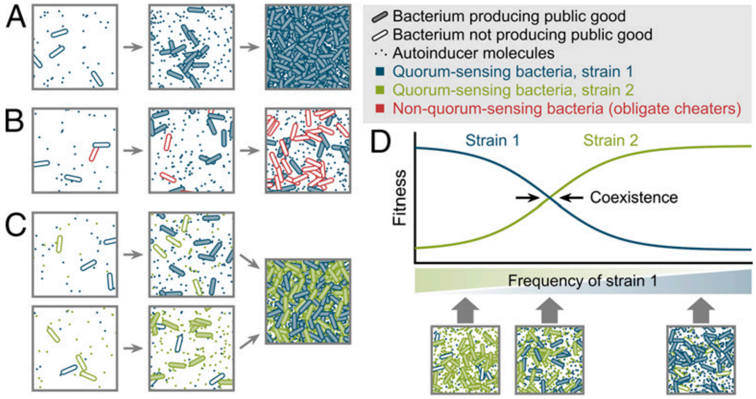

Fig. 1. Facultative cheating promotes the coexistence of different quorum-sensing mechanisms. (*A*) Growth of a population with a single strain of quorum-sensing bacteria (arrows represent time): at low frequencies, autoinducer concentration is too low to trigger the cooperative production of a public good (*Left*). When density is high enough, cooperation starts (*Center*). Cooperation benefits the group, allowing fast growth of the community up to high densities (*Right*). (*B*) Same as *A*, but in a mixed population with some cheater cells that do not respond to the autoinducer (red). Cheaters benefit from the public good produced by cooperators without contributing, thus growing in frequency. Because fewer cells contribute to the public good, the population grows more slowly than a pure culture of cooperators. (*C*) Same as *A*, but in mixed populations with two different strains of quorum-sensing bacteria (each strain responds only to its own autoinducer). (*Upper*) Starting condition with a minority of green cells. (*Lower*) Starting condition with a minority of blue cells. Both starting conditions lead to the same qualitative outcome: the minority strain acts as a cheater for some time, but eventually both strains reach enough density to trigger cooperation, and the population grows fast. (*D*) Fitness of each strain as a function of their relative frequency. Facultative cheating produces negative frequency-dependent selection (each strain has fitness advantage when at low frequency), leading to stable coexistence.

Quorum sensing is elegantly simple. The bacterium produces an autoinducer molecule that activates the cooperative mechanism. Unlike regular activation mechanisms, however, the autoinducer molecule does not stay inside the bacterium, but is instead secreted to the environment. If few bacteria are present, the autoinducer will diffuse away faster than it is produced, and the local concentration will be too low to activate cooperation. In contrast, a dense group of bacteria will accumulate enough autoinducer, triggering cooperation precisely when coordinated behavior is most effective (Fig. 1*A*) (4).

Similar to many other cooperative schemes, however, quorum sensing is vulnerable to cheaters (7). A mutant invader that ignores the autoinducer will benefit from the public good produced by cooperators, without contributing anything. The cheating mutant will grow faster than the wild-type, growing in frequency and hindering cooperation, so the population will grow more slowly (Fig. 1*B*).

In a paper published in PNAS in 2011, Avigdor Eldar predicted a different fate for an invader that carries an incompatible quorum-sensing machinery

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(8). Take the case of two different strains, blue and green, where each strain responds only to its own autoinducer (Fig. 1*C*). A minority of green cells will cheat in a blue population: when blue cells reach high enough density to trigger cooperation, green cells will benefit from it without contributing anything (they do not respond to blue autoinducer, and their own density is too low to accumulate green autoinducer). Green cells thus have a fitness advantage and increase in frequency, until their density is high enough for their own autoinducer to make them cooperate. In the long term, the population contains both strains, and both of them cooperate (Fig. 1*C*, *Upper*).

Green cells are therefore facultative cheaters: they cheat in some conditions (at low frequency) and not in others (at high frequency). However, note that blue cells can also cheat: if a blue cell enters a green population, it will free-ride on the cooperation of green cells for some time (Fig. 1*C*, *Lower*). Both strains are therefore facultative cheaters. This gives them a fitness advantage when at low frequency, and a disadvantage when at high frequency. This phenomenon, called negative frequency-dependent selection, leads to stable coexistence of both strains: whenever one strain's frequency drops, it free-rides on the other's cooperation, increasing in frequency and restoring the equilibrium (Fig. 1*D*).

Until today, this mechanism for coexistence was just theory in Eldar's paper (8). Now, along with Eldar, Pollak et al. present a beautiful experimental confirmation in *Bacillus subtilis* (6). This bacterium has a well-studied quorum sensing system, which regulates—among other things—the production of surfactin, a public-good molecule that facilitates swarming (9, 10). Pollak et al. (6) demonstrate the main predictions of the original model, including facultative cheating (as in Fig. 1*C*) and negative frequency-dependent selection (Fig. 1*D*).

Both facultative cheating and negative frequency-dependent selection are common in nature (11–13), but the system described by Pollak et al. (6) has some uncommon features. For example, it naturally allows for more than two coexisting strains: for any number of strains with different quorum-sensing mechanisms, each of them will cheat at low frequency, and all of them will—in theory—coexist. Pollak et al. show coexistence of only two strains, but they show pairwise negative frequency-dependent selection for four different strains. This result suggests that the same mechanism may allow coexistence of more than two strains.

Pollak et al. (6) find that cultures containing a single strain reach the same final densities as mixed cultures containing both strains. This is not a general property of negative frequency-dependent selection; in most systems, the stable configuration where both strains coexist has different collective fitness than the pure cultures. Perhaps different metrics of performance (e.g., growth rate rather than final density) would show a difference between mixed and pure cultures.

These results explain why diverse quorum-sensing mechanisms coexist. However, they do not explain how quorum-sensing systems are able to persist, given that obligate cheaters can invade the population. To explain persistence, we must resort to selection schemes that allow cooperators to interact with each other more than with cheaters (7, 14, 15). Pollak et al. use a scheme where several populations grow separately, are then pooled together, and a few cells are randomly chosen to seed new populations. Even if cheaters spread within each population, populations with a higher fraction of cooperators grow faster, contributing more cells to the next generation (7, 15). This selection scheme keeps obligate cheaters at bay, allowing quorum sensing to persist.

The incompatible quorum-sensing mechanisms serve as a kin discrimination system, where each individual recognizes whether other individuals are closely related and behaves differently with close relatives than with distant ones. Kin discrimination can help to maintain cooperation, because cooperators can cooperate only with close relatives (who are also cooperators), leaving cheaters out of the game (16). However, the kind of kin discrimination that emerges from quorum sensing works differently, because quorum-sensing bacteria cannot choose to cooperate only with close relatives; instead, when enough close relatives are around, bacteria produce a public good that benefits the entire population. Because of this difference, quorum sensing is vulnerable to obligate cheaters and needs special selection schemes to persist. In addition, classic kin discrimination favors homogeneous populations of closely related individuals that cooperate with each other and reject anybody else. In contrast, quorum sensing produces negative frequency-dependent selection, fostering diversity inside the population.

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The findings of Pollak et al. (6) suggest fascinating hypotheses for other species; the same principles should apply to any species that cooperates at high densities via public goods, and whose populations are genetically heterogeneous. Furthermore, similar principles might explain coexistence of different species: different bacterial species often share the same public goods and quorum-sensing mechanisms (4, 17, 18).

As we deepen our understanding of biological systems, we learn to see diversity not as an annoying noise in our measurements, but as a key feature of the system. Much of current research goes in this direction, from bet-hedging to behavioral interindividual variability (19, 20). Even in the midst of this paradigm shift, it seemed safe to assume that communication would require homogeneous populations, with all individuals using the same signals. As Pollak et al. (6) now show, the time has come to revisit this assumption.

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