Supporting Information

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SI Text

The aim of this section is to describe and analyze the mathematical model I use and to extend the discussion on the generality and impact of this unique evolutionary divergence mode. Below is a short summary of each of the sections, to help the reader focus on the material of interest.

i) Section 1 discusses the generality of my model of public goods regulation by quorum sensing. I bring examples for known secreted public goods in multiple QS systems and discuss other types of public goods and other functions of QS systems that are not related to public goods production. I also discuss the potential divergence of signals that do not measure "cell density" per se.

ii) Section 2 describes the development of the model, its rationale, and the way it is simplified into Eqs. 1–5 of *Materials and Methods* and used in the main text. I define the interaction matrices for various types of strain relations.

iii) Section 3 analyzes the steady-state behavior of a single cooperator strain and of two diverged cooperator strains. I show that the behavior of the system depends on a dimensionless factor that compares the rate of nutrient production by the enzyme and its rate of consumption through growth. Stability analysis of the coexistent steady-state solution of two strains shows that it is stable under all conditions.

iv) In section 4 I discuss the cost of signaling and its relation to the model. I claim that as all strains on the pathway for diversification produce a signal, its cost is not important for the process. I also discuss the difference between obligate cheater and inducer cheater with a functional signaling allele.

v) In section 5 I elaborate on the immunity of the novel cooperator to the intermediate cheater strain. I analytically demonstrate that in homogenous conditions the two strains maintain their frequency in the population and discuss the advantage of the induction to both strains in terms of total yield of the mixed population. I also discuss the inability of strain R_1S_2 to invade into the original wild-type QS system, R_1S_1.

vi) In section 6 I describe and analyze the bottleneck selection assay between cooperator and cheater. I analytically solve the problem for bottleneck sizes of one and two lineages and semianalytically show how to extend the analysis to larger bottleneck sizes. I specifically prove the advantages of the immune novel cooperator over the naive original cooperator in this competition.

vii) In section 7 I demonstrate that diversification still holds under more general models that extend the basic model in four important manners: (*i*) relaxing the assumption of full orthogonality between novel and original communication pathways, (*ii*) adding null alleles to receptor/signal/enzyme, (*iii*) assuming a positive feedback of receptor activity on receptor and signal expression levels, and (*iv*) assuming that the public goods have a greater benefit to the producer, leading to snowdrift dynamics.

viii) In section 8 I analyze the consequences of asymmetric interactions between strains. I first discuss the consequences of population structure on the evolution of cross-inhibition. I then show that asymmetric cross-activation can be a direct outcome of divergence and to benefit the signaling strain. I discuss the resulting arms race between strains and why it can lead to mutual cross-inhibition.

ix) In section 9 I compare previously described models for kin recognition with the kin-recognition system described in the text. Two main differences are emphasized: the use of two loci by the kin-recognition system and the decoupling of recognition and cooperation.

1. Quorum Sensing and Public Goods Production. A basic assumption of my model is that quorum sensing is controlling the production of "public goods"—products or behavioral strategies that benefit also cells whose QS system is nonfunctional. In this section I discuss the prevalence of public goods production under QS control and compare it with other functions of quorum sensing.

1.1. Quorum sensing and the regulation of secreted enzymes. As I mention in the main text, regulation of secretion is one of the major roles of quorum sensing. In fact, a recent review has suggested that the main use of the quorum sensing signal is to serve as a "cheaper" proxy to the fate of the more costly secreted molecules ("efficiency sensing") (1). Table S1 provides a set of examples for QS-dependent secreted molecules, including examples from the six known divergent quorum sensing systems.

1.2. Other types of public goods. Many other types of cooperation in bacteria can be regarded as producing public goods. This observation is not surprising, given the relatively simple ways by which bacteria can interact and the relative lack of direct cell–cell interactions in bacteria (but cf. refs. 2–4 for examples of direct contact interactions with obvious or presumed social impact). Here are two important types of public goods, which do not involve the secretion of molecules by the cells. Both of these types can lead to evolution of divergent QS systems in a similar manner to what is presented in the main text:

a) Removal of public bad: Enzymes that intracellularly degrade toxins help other cells by continuously removing the toxin from the environment. An example of this behavior is the formation of satellite colonies sensitive to beta-lactam antibiotics next to a colony expressing the beta-lactamase resistance gene (which is expressed in the periplasm). I note that it is not clear whether there is any advantage for regulating these types of anti–public-bad enzymes by QS, as the public-bad presence may be independent of density. If toxin is constitutive, then a QS regulation is not likely.

b) Restrictive growth: Under various conditions, cooperating cells may favor a mode of growth that restricts their growth rate to avoid reduced yield (5) or to prevent disruption of spatial architectures (6). The public good here is the growth potential, which cells are choosing not to consume at maximal possible rate. It is not known whether quorum sensing actually regulates such a behavior, but there has been very little work in this direction.

c) Altruistic "suicide": In the case where part of the population "decides" to dedicate its behavior toward the benefit of other cells and not toward reproduction, it is effectively creating a public good. This behavior may not always lead to secretion of specific molecules. For example, in the production of a fruiting body, the stalk cells are altruistic suicides. Many fruiting body cheaters are strains that do not invest in stalk cells. Here the public good is the work done by the altruists toward the rest of the community. There are various extracellular signaling pathways working during

fruiting body development in both bacteria and amoebas and recent work indicates that some of the social mutants in amoebas are related to signaling (7).

d) Symbiosis: The production of a material that benefits a symbiosis partner that in turn benefits the community is an indirect public good. The best studied case is the symbiosis between the bacteria *Vibrio fischeri* and the Hawaiian bobtail squid, *Euprymna scolopes*. Here, QS drives the expression of the lux enzymes that catalyze the production of light, helping the squid. The squid, in return, provides food and a controlled environment for the bacteria. It was shown that both QS and lux mutants are outcompeted by wild type when competing in juvenile squids (8, 9). Also, "dark" strains were never isolated from squid-hosted bacterial populations (10). It is not clear yet what is the mechanism of selection, although it may involve both a benefit to light producers and punishment to light nonproducers (the lux gene reduces reactive oxygen species introduced by the squid) (8). In both cases, a highly structured population imposed by the squid is probably an important part of the selection against dark cheaters. There are no reports on diversification of signal in the *V. fischeri* lux system.

1.3. Divergence under "nonquorum" signals. The simple model of quorum sensing described here assumes that signal production is constitutive and therefore that the activation of quorum response is density dependent. In other cases, signaling may be regulated and activation may depend not only on density but also on other characteristics of the population. I emphasize that the model discussed in this paper is valid also for this extended type of signaling. The important criterion is the regulation of public goods and not the conditions under which public goods are produced. Nonquorum signals may be specifically important in complex behaviors, such as fruiting body formation and biofilm formation.

1.4. Quorum signal as a public good. The QS signal is costly to produce and leads to a benefit to all receiving cells; therefore it is a public good. However, this public good cannot lead to the divergence of the quorum sensing system. From the perspective of the quorum sensing system, signal is a "club good" (11)—it can be used only by cells with an appropriate receptor and therefore cells with a divergent receptor will not be able to be cheaters (see next section). See more discussion on the cost of signaling in *SI Text*, section 4.

1.5. Functions of quorum sensing that are not related to public goods. Multiple functions of quorum sensing do not follow the definition of public goods. In general, these functions can be divided into two categories:

a) Single-cell use: It has been postulated that QS has actually evolved as a mechanism for probing the environment by single cells ("diffusion sensing") (12). Relevant biological examples are the invasion of bacteria into host cells and other cases where environment is highly compartmentalized. Whereas it is highly controversial whether this view is correct (and the subject of this paper—QS diversification—is most likely a proof to the contrary of this view), it may very well be that in some species diffusion sensing or "confinement sensing" is the main function of a QS system.

b) Private goods and club goods: Many other QS functions may lead to the benefit of sensing cells only, either because they do not impact non-QS cells ("private goods") or because only QS cells can take advantage of their utility (club goods). Here are examples for the two types:

i) Private goods: Certain species of bacteria will use QS to regulate the dispersal of complex structures, like biofilms. In this case non-QS cells will simply not disperse and will not be able to enjoy the benefits of dispersal. Another example is the utilization of QS for genetic transmission as occurs in various cases, both at the level of the bacteria (genetic competence systems) and at the level of various selfish genetic systems (conjugation, etc.).

ii) Club goods: Many species of bacteria produce a coupled set of products—a secreted product and a cell-autonomous product that is necessary for the utilization of the secreted product. If both products are under the control of QS, then QS mutants will not be able to use the product secreted by others. This form of club goods includes siderophore–receptor pairs—if both are under QS control, then a QS null strain will not be able to use the siderophore. Another example is antibiotic-resistance pairs. Here, the QS null mutant cells may be killed by the QS-active cells.

Note that whereas QS-regulated club goods seem to be a good mechanism for preventing the cheating of quorum sensing systems, they have a major drawback—they also prevent the ability to act as a facultative cheater on the secreted product—to enjoy it when it is produced by other strains at low cell densities. This drawback is most likely the reason why both siderophore receptors and immunity genes have additional modes of regulation apart from QS.

2. A Mathematical Model for QS System Interaction. As described in the main text, I assume that a quorum sensing signaling pathway regulates the production of a public good—an exo-enzyme in this case—whose production is costly, but is necessary for growth. Specifically, I assume that the exo-enzyme (E) catalyzes the cleavage of a complex nutrient (P) (e.g., a sugar polymer) into a transportable form (P_d) (e.g., a sugar monomer). I assume that the level of complex nutrient is constant. Other variables I use in the model are the receptor level (R), the signal level (S), the receptor–signal complex ($[RS]$), and cell density (n). To formulate a model of such a system I make a set of specific assumptions. The nature of my hypothesis is such that it will most likely be true in many other models of public goods controlled by quorum sensing, as long as QS null mutants can invade the wild type. Some of these extensions are further explored in *SI Text*, sections 7 and 8.

2.1. A model for a single strain. The specific assumptions I make here are as follows:

i) Growth is dependent (through a Holling's type II term) on the nutrient levels.

ii) A fraction r of the growth potential is diverted from growth to enzyme production in the quorum responding cell at the maximal production level.

iii) Cells may die in a density-dependent manner (logistic growth) or, in low probability, spontaneously.

iv) Enzyme production is a function of a signal molecule bound receptor.

v) Complex nutrient levels are large compared with the affinity of the exo-enzyme.

vi) Signal molecule productions and receptor production are constitutive.

These assumptions fit the following set of equations:

$$\frac{d\tilde{n}}{dt}=\left(\alpha_n\frac{\tilde{p}_d}{\tilde{p}_d+K_G}(1-rf([RS]))-\beta_n\tilde{n}-\tilde{\gamma}_n\right)\tilde{n}\quad\text{Cell density}\quad\textbf{[S1]}$$

$$\frac{d\tilde{S}}{dt}=P_S\tilde{n}-\tilde{\beta}_S\tilde{S}-\tilde{n}(k_+R\tilde{S}-k_-[RS])\quad\text{Signal}\quad\textbf{[S2]}$$

$$\frac{dR}{dt} = P_R - k_+ R\tilde{S} + k_- [RS] - \beta_R R \quad \text{Receptor} \quad \textbf{[S3]}$$

$$\frac{d[RS]}{dt} = k_+ R\tilde{S} - k_- [RS] \quad \text{Receptor-signal complex} \quad \textbf{[S4]}$$

$$\frac{d\tilde{E}}{dt} = P_E f([RS])\tilde{n} - \tilde{\beta}_E \tilde{E} \quad \text{Enzyme} \quad \textbf{[S5]}$$

$$\frac{d\tilde{P}_d}{dt} = \tilde{J}_{P_d} + \tilde{V}_{\max}\tilde{E} - \tilde{\beta}_{P_d}\frac{\tilde{P}_d}{\tilde{P}_d + K_{P_d}} n \quad \text{Nutrient} \quad \textbf{[S6]}$$

I further assume that the quorum response function $f([RS])$ is a monotonous increasing function with maximum of 1.

I can now simplify the set of equations and the number of parameters by normalizing some of the parameters and assuming several simplifying assumptions. The only parameters that differ between the strains in the system are those of the quorum response, $f([RS])$, which I do not normalize and describe explicitly in the following subsection. Simplifying assumptions include the following:

Timescale: This is set by the maximal growth rate, $\alpha_n = 1$.

Population density is measured by units of $\frac{\alpha_n}{\beta_n}; n = \frac{\beta n}{\alpha_n}\tilde{n}$.

Signal molecule concentration is measured in units of $P_S/\beta_S; S = \frac{\tilde{S}}{P_S/\beta_S}$.

Enzyme levels are measured by units of $P_E/\beta_n; E = \frac{\tilde{E}}{P_E/\beta_n}$.

I assume that the interaction between signal molecule and receptor happens on a much faster timescale than others, so it is in quasi-steady state, and that the levels of receptor are constant. This assumption implies a Michaelis–Menten relationship between $[RS]$ and S: $[RS] = \frac{S}{K_{RS} + S}$.

Nutrient concentration is normalized by their growth saturation value; $P_d = \frac{\tilde{P}_d}{K_{P_d}}$.

These assumptions and normalizations lead to the following set of equations:

$$\frac{dn}{dt} = \left(\frac{P_d}{P_d + 1}\left(1 - rf\left(\frac{S}{K_{RS+S}}\right)\right) - n - \gamma_n\right)n \quad \text{Cell density} \quad \textbf{[S7]}$$

$$\frac{ds}{dt} = \beta_S(n - S) \quad \text{Signal} \quad \textbf{[S8]}$$

$$\frac{dE}{dt} = f\left(\frac{S}{K_{RS+S}}\right)n - \beta_E E \quad \text{Enzyme} \quad \textbf{[S9]}$$

$$\frac{dP_d}{dt} = J_{P_d} + V_{\max}E - \beta_{P_d}\frac{P_d}{P_d + 1}n \quad \text{Nutrient,} \quad \textbf{[S10]}$$

where the remaining parameters are appropriately normalized: $\left(\gamma_n = \frac{\tilde{\gamma}_n}{\alpha_n}; \beta_S = \frac{\tilde{\beta}_S}{\alpha_n}; \beta_E = \frac{\tilde{\beta}_E}{\alpha_n}; J_{P_d} = \frac{\tilde{J}_{P_d}}{K_{P_d}\alpha_n}; V_{\max} = \frac{\tilde{V}_{\max}P_E}{\beta_n \alpha_n K_{P_d}}; \beta_{P_d} = \frac{\tilde{\beta}_{P_d}}{K_{P_d}\beta_n}\right)$. For the quorum response form, I use $f(x) = x^m$. The initial levels of all variables except for cell density are set to zero.

I note here that the expression of the QS system constitutes a physiological positive feedback—the more cells there are, more QS signal and public goods are made and there is more potential for growth. This feedback may lead to threshold dependence on parameters. This positive feedback is not directly related to the molecular positive feedback often found in QS systems—the quorum response activating the expression of signal and receptor.

2.2. The effect of multiple strains with varying signals on receptor activity. To analyze the interaction between different strains, I need to define the type of interactions between various signals and receptors. To allow for inhibiting interactions as well as activating ones, I assume that a QS receptor has two states, active prone (R_i^{ac}) and inactive prone (R_i^{in}). I assume a simple form of competition for the two states of the receptor:

$$R_i^{\text{ac}} + S_j \leftrightarrow R_i^{\text{ac}} S_j \quad \textbf{[S11]}$$

$$R_i^{\text{in}} + S_j \leftrightarrow R_i^{\text{in}} S_j \quad \textbf{[S12]}$$

$$R_i^{\text{in}} \leftrightarrow R_i^{\text{ac}}. \quad \textbf{[S13]}$$

It can be easily shown that this leads to a quasi-steady state of the active receptor–signal molecule complex of the form

$$\begin{aligned} R_j^{\text{active}} &= R_j^{\text{tot}} \frac{\sum_i K_{ij}^{\text{ac}} S_i}{\left(K_{RS} + \sum_i K_{ij}^{\text{ac}} S_i + \sum_i K_{ij}^{\text{in}} S_i\right)} \\ &= R_j^{\text{tot}} \frac{K^{\text{ac}} S}{\left(K_{RS} + K^{\text{ac}} S + K^{\text{in}} S\right)}, \end{aligned} \quad \textbf{[S14]}$$

where K^{ac} and K^{in} are the two matrices that define activatory and inhibitory interactions of the strains through the signals. Some examples for the matrices defining the relations discussed in the paper are as follows:

i) R_1S_1 strain vs. R_2S_2 strain (facultative cheaters): $K^{\text{ac}} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}$

ii) R_1S_1 strain vs. R_0S_0 strain (obligate cheater, complete): $K^{\text{ac}} = \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix}$

iii) R_1S_1 strain vs. R_2S_1 strain (obligate cheater, signal producer): $K^{\text{ac}} = \begin{pmatrix} 1 & 1 \\ 0 & 0 \end{pmatrix}$

iv) R_2S_1 strain vs. R_2S_2 strain (strain 2 is an immune cooperator activating strain 1): $K^{\text{ac}} = \begin{pmatrix} 0 & 1 \\ 0 & 1 \end{pmatrix}$

v) Asymmetric cross-inhibition is represented by $K^{\text{in}} = \begin{pmatrix} 0 & 1 \\ 0 & 0 \end{pmatrix}$, whereas the symmetric one is represented by $K^{\text{in}} = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}$.

In *SI Text*, section 7, I consider the effects of a nonorthogonal cross-activation term on the evolution of divergence. In this case, all zeros in the matrices shown in examples *ii*–*iv* should be replaced with $0 < \rho < 1$.

2.3. Competition between two strains. To analyze the competition between two strains, I use the same equations as in the single-strain model (Eqs. **S7**–**S10**), but dedicate specific equations to the cell density and signal of each strain. This method results in the equations presented *Materials and Methods* in the main text:

$$\frac{dn_i}{dt}=\left(\frac{P_d}{P_d+1}\left(1-rf\left(R_i^{\text{active}}\right)\right)-n_{\text{tot}}-\gamma_n\right)n_i,\quad i=1,2,\ldots,N \tag{S15}$$

$$\frac{dS_i}{dt}=\beta_S(n_i-S_i),\ i=1,2,\ldots,N \tag{S16}$$

$$\frac{dE}{dt}=\sum f\left(R_i^{\text{active}}\right)n_i-\beta_E E \tag{S17}$$

$$\frac{dP_d}{dt}=J_{P_d}+V_{\max}E-\beta_{P_d}\frac{P_d}{P_d+1}n_{\text{tot}}. \tag{S18}$$

Two additional variables whose time evolution is useful to follow when considering social invasion patterns are $n_{\text{tot}}=n_1+n_2$ and $s=\frac{n_1}{n_2}$. I can easily find that

$$\begin{aligned}\frac{dn_{\text{tot}}}{dt}=&\left(\frac{P_d}{P_d+1}\left(1-rf\left(R_1^{\text{active}}\right)\right)-n_{\text{tot}}-\gamma_n\right)n_{\text{tot}}\\&+r\frac{P_d}{P_d+1}\left(f\left(R_2^{\text{active}}\right)-f\left(R_1^{\text{active}}\right)\right)n_2\end{aligned} \tag{S19}$$

$$\frac{ds}{dt}=n_2^{-2}\left[n_1\frac{dn_2}{dt}-n_2\frac{dn_1}{dt}\right]=r\frac{P_d}{P_d+1}\left(f\left(R_2^{\text{active}}\right)-f\left(R_1^{\text{active}}\right)\right)s. \tag{S20}$$

Therefore, total cell density behaves as a single strain with an additional term for the difference in produced public goods between the strains. The change in relative frequency of the two strains is directly proportional to their public goods production difference due to its impact on the growth rate. Therefore, any steady state will be reached only when both strains produce the same level of public goods. This result is intuitively clear, as only then cost and benefit will be balanced in the same way.

Note that the density-dependent death is proportional to n_{tot} as is commonly used in logistic growth models. The reason for assuming density-dependent death is to keep the steady-state dynamic. In models of growth by expansion [like the x–y expansion of swarming models (13) or the z expansion of biofilm models (14, 15)], this term is most likely unnecessary.

2.4. A QS null mutant is a cheater in the model. A basic aspect of a public goods model is that a strain that is not producing the public goods will be deficient on its own, but will be able to invade a community of a wild-type public goods-producing population. This process, however, will lead to a reduction in the final population fitness as public goods levels are diminished. This result is demonstrated for the model presented in Eqs. **S15**–**S18** in Fig. S1; see the Fig. S1 legend for details.

Interaction between obligate cheater (strain 2) and cooperator (strain 1) is described by the interaction matrix $K^{\text{ac}}=\begin{pmatrix}1&0\\0&0\end{pmatrix}$. I can easily see that the cheater will invade the population from Eq. **S20**. As cheaters do not contribute to public goods production, I find that

$$\frac{ds}{dt}=-r\frac{P_d}{P_d+1}f\left(R_1^{\text{active}}\right)s<0 \tag{S21}$$

and therefore cooperator levels will always decrease.

3. Steady-State Analysis. ***3.1. Single strain.*** A steady-state cell density and signaling level will be achieved as long as the level of usable nutrient, P_d, is either constant or high enough to sustain maximal growth. Three different limiting factors will lead to a different behavior:

i) Growth-limited regime: Nutrient production by the exoenzyme is larger than its maximal consumption ($P_d\gg 1$).
ii) Enzyme-limiting regime: Enzyme levels are such that they do not allow maximal growth.
iii) Signal-limiting regime: Signaling does not lead to maximal production of enzyme.

3.1.1. Case 1—growth limited. If $P_d\gg 1$, the quasi–steady-state equations for cell density are reduced to

$$0=\left(\left(1-rf\left(\frac{S}{K_{RS}+S}\right)\right)-n-\gamma_n\right)n \tag{S22}$$

$$0=\beta_S(n-S), \tag{S23}$$

which yield

$$n=\left(1-rf\left(\frac{n}{K_{RS}+n}\right)\right)-\gamma_n\ \ (\text{or}\ \ n=0). \tag{S24}$$

If $n>K_{RX}$ (i.e., signal is saturating the receptor), I find that $n=1-r-\gamma_n$. In this case, the cells reach the highest possible density of enzyme-producing cells.

To attain this approximation, the enzyme production rate needs to be high enough to maintain $P_d\gg 1$, which implies that the P_d formation rate is faster than its use (Eq. **S18**):

$$V_{\max}E>\beta_{P_d}n. \tag{S25}$$

Using Eqs. **S7**–**S10** in steady state, I find that this result is equivalent to

$$\zeta\equiv\frac{V_{\max}}{\beta_{P_d}\beta_E}>1. \tag{S26}$$

3.1.2. Case 2—enzyme-limited growth. If $P_d<1$, growth is restricted by the enzymatic conversion rate, and I can calculate the steady state from Eqs. **S7**–**S10**:

$$n=\zeta\left(1-rf\left(\frac{n}{K_{RS}+n}\right)\right)f\left(\frac{n}{K_{RS}+n}\right)-\gamma_n. \tag{S27}$$

Again, if $n>K_{RS}$, I find

$$n=\zeta(1-r)-\gamma_n. \tag{S28}$$

The two cases demonstrate that the critical parameter that determines the nature of quasi-steady state if signaling is saturated is

$$\zeta=\frac{V_{\max}}{\beta_{P_d}\beta_E}=\frac{\tilde{V}_{\max}\left(\frac{P_E}{\beta_E}\right)}{\tilde{\beta}_{P_d}}. \tag{S29}$$

This dimensional parameter represents the relative level of nutrient production and consumption in steady state. The second critical parameter is K_{RS}, which allows efficient utilization of the signal. In an ideal scenario, enzyme production will be tuned such that its value is optimal for growth, which depends on the exact level of enzyme cost. Under most scenarios this level will be close to $\zeta=1$, which is the level chosen for the simulations in the main text.

Note that in the above analysis I neglected the constant usable nutrient production term J_{P_d}. In the simulations I performed, I assume this term to have a small, but nonzero, level. This assumption implies that there is a lower limit to cell density, even if

no enzyme is produced, thereby setting a nonzero fitness to a cheater-only population.

3.1.3. Case 3—signal-limited growth. What happens if signal does not saturate the receptor and enzyme's production is not maximal? I assume $f(x) = x$. If $K_{RS} > n$, I find

$$\begin{aligned} n &\cong \zeta\left(1-r\frac{n}{K_{RS}+n}\right)\frac{n}{K_{RS}+n}-\gamma_n \Rightarrow 1 \\ &\cong \zeta\left(1-r\frac{y}{1+y}\right)\frac{1}{1+y}\frac{n}{K_{RS}} \cong \frac{\zeta(1-r)}{K_{RS}}-1. \end{aligned} \quad \text{[S30]}$$

If $K_{RS} > \zeta(1-r)$, then this result will not yield a positive steady state and cell density will approach 0. If $\zeta(1-r) \gg K_{RS}$, then the signal becomes saturated. The signal-limited situation occurs therefore only in a small region of parameter space where $\zeta \sim K_{RS}$. Fig. S2 shows the steady-state level of cells for the different values of ζ, K_{RS}.

3.2. Competing strains. I consider here the case of two competing strains with functional and divergent QS systems having the same parameters. It is clear that as both strains have equivalent QS systems, the steady state is symmetric (assuming a unique steady state):

$$n_1 = n_2 = \frac{n_{\text{tot}}}{2}. \quad \text{[S31]}$$

If I use this relation in Eqs. **S15**–**S18** and assume a steady state, I find the exact two solutions found for the single-strain steady state, but now with $\frac{n_{\text{tot}}}{2}$ appearing in the expression for f:

$$\text{Case 1:}\quad n_{\text{tot}} = \left(1-rf\left(\frac{\frac{n_{\text{tot}}}{2}}{K_{RS}+\frac{n_{\text{tot}}}{2}}\right)\right)-\gamma_n \quad \text{[S32]}$$

$$\text{Case 2:}\quad n_{\text{tot}} = \frac{V_{\max}}{\beta_{P_d}\beta_E}\left(1-rf\left(\frac{\frac{n_{\text{tot}}}{2}}{K_{RS}+\frac{n_{\text{tot}}}{2}}\right)\right)f\left(\frac{\frac{n_{\text{tot}}}{2}}{K_{RS}+\frac{n_{\text{tot}}}{2}}\right)-\gamma_n. \quad \text{[S33]}$$

In both cases, if signal levels are sufficiently high to saturate the receptors, then the steady-state levels of the mixed culture are close to the one for the pure culture. In case 1, it may actually be slightly higher, as enzymes are overproduced by the single strain and enzyme cost is slightly reduced in the mixed population. A similar scenario was recently suggested in an experimental cooperative system (16).

3.2.1. Stability analysis. To address the stability and uniqueness of the equal-concentration steady state, Eq. **S31**, I analyze the relative levels of n_1, n_2. I define $s = \frac{n_1}{n_2}$. Using Eq. **S20**, I find that if $n_1 > n_2$, the time derivative of s follows

$$\frac{ds}{dt} = r\frac{P_d}{P_d+1}\left(f\left(R_2^{\text{active}}\right)-f\left(R_1^{\text{active}}\right)\right)s < 0. \quad \text{[S34]}$$

This result is true because R is a monotonic increasing function of n and f is a monotonic increasing function of R. The inequality implies that the relative frequency of n_2 increases when it is in a minority and decreases when in a majority, implying that the steady-state $n_1 = n_2$ is stable.

4. A Note on the Cost of Signaling. A quorum-sensing signal is by itself a type of public good and it was experimentally shown that a signal-deleted strain will be able to invade into a wild-type population in both natural and synthetic contexts (17, 18). Although true in general, the cost of signaling does not affect the diversification of quorum-sensing systems. This is so because all relevant strains (R_1S_1, R_2S_1, R_2S_2, and R_1S_2) contain a secreted signal and therefore pay the cost of signaling. The difference between the strains is only in the effectiveness of the signal in a specific social context, not its cost.

It is worthwhile to note few related complications:

i) An extended model has to include also null alleles of receptor, signal, and enzyme. In *SI Text*, section 7 I show that such an extension does not interfere with either the evolution of an intermediate cheater or the evolution of an immune cooperator.

ii) In natural quorum-sensing systems, the cost of quorum response is larger than the cost of signal production, as was shown in *Pseudomonas aeruginosa* (17). This result implies that signal allele mutants are more weakly selected. In fact, a recent paper demonstrated conditions under which receptor null mutants are easily selected and signaling null mutants are not selected at all (19).

iii) Also, as noted in ref. 17, if a positive feedback on signaling is established through the quorum response, then most of the signaling cost is actually included in the cost of quorum response. It is only the constitutive signal production that is not included. Whereas QS cannot diverge if the constitutive signal is the only public good in the system (*SI Text*, section 1), QS divergence can be selected if QS directs the production of signal (forming a positive feedback on signal production).

Finally, it is worth noting that the strain R_2S_1 is both a "cheater" and a "liar". It better exploits cooperators, by inducing them to produce the enzyme. This additional function can be seen in the longer invasion of the strain R_0S_1 compared with a full QS mutant R_0S_0 in Fig. S3 (0 refers to a null mutant). It is easy to understand why "induction" is not a significant contribution to cheating—when inducer–cheater levels are low, the effect of the signal they produce is insignificant. If they are high, then there are no cells to induce. Therefore, an inducer–cheater will have a marginal advantage over a "silent" cheater only for intermediate occupancy. This outcome might be balanced off by the cost of constitutive signal production. If the signal is under positive feedback through the quorum response, than this effect will be even smaller (17) as the cheater strain is producing only low levels of signal.

5. Immunity to Cheating by Cooperation Induction. As explained in the main text, the novel cooperator, R_2S_2, is immune to the cheating of its ancestor, R_2S_1, by inducing its quorum response. I show that the immune cooperator strategy is neutral in a mixture with the ancestor: Its frequency does not change with time. I also show that the community benefits from it, as some public good is produced by both strains and therefore the total cell density reached is higher than in a pure cheater (R_2S_1) population. I further discuss these properties and some of their consequences in this section.

5.1. The immune cooperator phenotype in homogenous conditions. I consider the mathematical implication of competition between the immune cooperator, R_2S_2, and the intermediate strain R_2S_1. This competition can be analyzed using Eqs. **S15**–**S20** with the activation matrix $K_{ij}^{\text{ac}} = \begin{pmatrix} 1 & 0 \\ 1 & 0 \end{pmatrix}$. As the receptors in both strains are the same, both depend on $f(R_1^{\text{active}}) = f(R_2^{\text{active}})$. I find

$$\frac{dn_{\text{tot}}}{dt} = \left(\frac{P_d}{P_d+1}\left(1-rf\left(R_1^{\text{active}}\right)\right)-n_{\text{tot}}-\gamma_n\right)n_{\text{tot}} \quad \text{[S35]}$$

$$\frac{d\left(\frac{n_2}{n_1}\right)}{dt} \propto f\left(R_2^{\text{active}}\right)-f\left(R_1^{\text{active}}\right)=0 \quad \text{[S36]}$$

$$\frac{dS_1}{dt} = \beta_S\left(sn_{\text{tot}}-S\right) \quad \text{[S37]}$$

$$\frac{dE}{dt} = f\left(R_1^{\text{active}}\right)n_{\text{tot}}-\beta_E E \quad \text{[S38]}$$

$$\frac{dP_d}{dt} = V_{\max}E-\beta_{P_d}\frac{P_d}{P_d+1}n_{\text{tot}}. \quad \text{[S39]}$$

The first characteristic of the immune cooperator is an immediate consequence of Eq. **S36**; as $\frac{ds}{dt}=0$, the initial fraction of the immune cooperator will remain constant along growth. This basic result stems from the fact that both cost and benefit are shared by the two strains and therefore the cooperator strain has no selective advantage or disadvantage. Therefore, in homogenous conditions, the immune cooperator is able to invade into the cheater only by neutral drift. However, I note that Eqs. **S35–S39** are equivalent to Eqs. **S7–S10** for the single-strain dynamics with the only difference that signal production is s times lower. This result implies that the strains will reach a nonzero steady state with a total density

$$\eta_{\text{tot}} \cong \zeta(1-r)-\gamma_n \quad \zeta<1 \quad \text{[S40]}$$

$$\eta_{\text{tot}} \cong (1-r)-\gamma_n \quad \zeta>1, \quad \text{[S41]}$$

but with $\zeta=\frac{V_{\max}}{\beta_{P_d}\beta_E}s$. Therefore, the higher the immune cooperator's abundance is, the higher will be the total cell density, as it induces more benefits to the population as a whole. As I show in *SI Text*, section 6, the two conditions needed for the fixation of the immune cooperator in a structured population when in competition with the intermediate cheater are the constancy of frequency and the monotonous increase in the total cell density with the cooperator's frequency in unstructured populations. As I show above, both of these conditions are met.

I note here that the immune cooperator's advantage is mainly apparent once it reaches a quorum. Therefore, there will typically be an almost-neutral phase in the evolution of the cooperator, where a single mutant cell is sufficiently multiplied to start activating the quorum response. This effect will be more pronounced in a quorum-sensing model with a threshold behavior of quorum response, as the cell gets very little benefit before its density/number is sufficiently high. However, the size of a quorum in real-life context can be fairly small (depending on the geometry and diffusion characteristics of the signal), so this "almost-neutral" drift phase may not be very large.

6. Comparing Cooperation Maintenance of Naive and Immune Cooperator Strains Under Population Bottlenecks. Previously, Griffin et al. (20) and similarly Chuang et al. (18) used a simple experimental assay to assess the maintenance of cooperation under four different ecological regimes involving low and high relatedness in cooperativity and local and global competition between lineages. The experimental procedure they devised is simple and useful for getting simple insight into the social interaction between strains. In this section, I generalize this approach to arbitrary levels of relatedness.

I use this assay to show that the immune cooperator, R_2S_2, will always be fixed when mixed with the intermediate cheater strain R_2S_1 irrespective of the level of relatedness. Therefore, it will always do better than the naive cooperator, R_1S_1, when competing with the intermediate strain, R_2S_1.

The assay I simulate is the following (Fig. S4 *A* and *B*, adapted from ref. 21): I assume there are a very large number, M, of populations (e.g., different tubes):

i) For N_s number of strains, I initiate the M populations, by randomly choosing N cells (bottleneck size) from the different strains with equal probability. That is, I initially assume that the abundance of all strains is equal.
ii) Growth: Each population develops according to Eqs. **S10–S13** for a time τ.
iii) Selection:
  a) Local competition: N cells are randomly chosen from each grown population and seed the next cycle of growth in this population.
  b) Global competition: All M populations are mixed together. Each population is reseeded by N randomly drawn cells from the population mixture.
iv) Cycle back to stage *ii*.

The characteristic parameter I am interested in is the asymptotic frequency of the different strains in the population. Specifically, I am interested in the outcome of this selection scheme for the competition between two strains: a cooperator and a cheater. I denote the frequency of cooperators in this assay as $F(N)$ [the frequency of cheaters is therefore $1-F(N)$]. For a population with bottleneck size N, I can calculate this value on the basis of a $(N+1)\times 2$ matrix n_{ij} representing the density of cooperators ($j=1$) and cheaters ($j=2$) in a mixed population after growth for a time τ, when seeded with $i=0,1,\ldots,N$ cooperator cells. (Note that cooperator levels at $i=0$ and cheater levels at $i=N$ are identically zero, reducing the number of free variables to $2N$.) I can express these parameters using the functions $n_{\text{tot}}(x)$, $0<f_c(x)<1$, the total cell density and the frequency of cooperators at the end of the growth phase when the initial frequency is x:

$$n_{i1}=n_{\text{tot}}\left(\frac{i}{N}\right)f_c\left(\frac{i}{N}\right);\quad n_{i2}=n_{\text{tot}}\left(\frac{i}{N}\right)\left(1-f_c\left(\frac{i}{N}\right)\right). \quad \text{[S42]}$$

The invasion of the cheater strain implies that the cooperator frequency always decreases with time during growth; therefore for $x\neq 1$ I find the relation

$$f_c(x)\leq x. \quad \text{[S43]}$$

Equality is true for the immune cooperator (*SI Text*, section 5), whereas a naive cooperator will follow the strong inequality.

In the following sections I analyze the asymptotic frequency of cooperators for local (F_l) and global (F_g) selection types. I first discuss the simple cases $n=1$, 2 used by ref. 20, and I then derive a general formula for arbitrary N and use it to show that the immune cooperator's frequency is always maximal for the specific competition type (1 for global and $\frac{1}{2}$ for local competition) irrespective of bottleneck size, whereas the frequency of the naive cooperator will always go below 1 for a large enough bottleneck size during global competition.

6.1. Local competition. n = 1. In local competition each population is seeded by its population from the previous culture. For the case $n=1$, this simply means that populations of cheaters will be kept as cheaters and populations of cooperators as cooperators, im-

plying that on average it will always be equal to the initial drawing probability, $F_1(0) = 0.5$.

n = 2. Each test tube is initiated with two random cells and grown for a time τ. There are now three types of cultures, two pure and one mixed. If a test tube is pure it will remain pure, whereas if it is a mix, it will diverge into a series of pure and mixed test tubes with ratios that depend on the relative frequencies of pairs chosen from the mixed population. One can show that the number of mixed tubes will always go down with time (N_{mix}, N_{coop}, and N_{cheat} denote the fractions of mixed tubes, pure cooperator tubes, and pure cheaters tubes):

$$N_{mix}(k+1) = \frac{2n_{11}n_{12}}{(n_{11}+n_{12})^2}N_{mix}(k) \Rightarrow N_{mix}(k) = N_{mix}(0)\left(\frac{2n_{11}n_{12}}{(n_{11}+n_{12})^2}\right)^k \quad \text{[S44]}$$

$$N_{coop}(k+1) = N_{coop}(k) + \frac{n_{11}^2}{(n_{11}+n_{12})^2}N_{mix}(k) \quad \text{[S45]}$$

$$N_{cheat}(k+1) = N_{cheat}(k) + \frac{n_{12}^2}{(n_{11}+n_{12})^2}N_{mix}(k). \quad \text{[S46]}$$

One can show that these relations imply that at the limit

$$N_{coop}(\infty) = N_{coop}(0) + \frac{n_{11}^2}{(n_{11}^2+n_{12}^2)}N_{mix}(0). \quad \text{[S47]}$$

In the above design, the initial levels are $N_{mix}(0) = 0.5, N_{cheat}(0) = 0.25$, $N_{coop}(0) = 0.25$ and I find

$$F_1(2) = N_c(\infty) = 0.25 + \frac{n_{11}^2}{(n_{11}^2+n_{12}^2)}N_{mix}(0). \quad \text{[S48]}$$

For the naive cooperator I typically get $n_{11} \ll n_{12}$, implying that $F_1^{Naive}(2) \sim 0.25$. For the immune cooperator, I find $F_1^{immune}(2) = 0.5$.

General N. Again, pure cooperators and cheaters will always be maintained whereas mixed tubes will be eliminated. I define M_k as the fraction of tubes initiated from k cooperators and find that the change in this fraction from one iteration to the next depends on a transition matrix $R_{k\to r}$, which defines the chances of getting r cooperators after a growth experiment that was initiated with k cooperators:

$$M_r(i+1) = \sum_{k=0}^{N} M_k(i)P_{k\to r};\ P_{k\to r} = \binom{N}{r}\rho^r(1-\rho)^{N-r};\ \rho = \frac{n_{k_1}}{n_{k_1}+n_{k_2}}. \quad \text{[S49]}$$

Or, in a shorter notation

$$\vec{M}(i+1) = R\vec{M}(i), \quad \text{[S50]}$$

where R is the transition matrix and M is the vector of fractions.

What do I know about $R_{k\to r}$? That both cheater and cooperator pure states are attractors,

$$R\mathbf{e_0} = \mathbf{e_0},\ \ \mathbf{e_0} = (10\ldots0)^T \quad \text{[S51]}$$

$$R\mathbf{e_M} = \mathbf{e_M},\ \ \mathbf{e_M} = (10\ldots0)^T. \quad \text{[S52]}$$

This condition implies that the matrix R has an eigenvalue of 1 with degeneracy of 2. As none of the other mixed states is an attractor—pure states will always form from any intermediate mixed state—the other eigenvalues must be <1:

$$R = U\lambda U^{-1} = U\begin{pmatrix} 1 & \ldots & 0 \\ & \lambda_1 & \\ \vdots & & \lambda_2 \\ & & & \ddots \\ 0 & \ldots & 1 \end{pmatrix}U^{-1}, \lambda_i < 1 \quad \text{for } i = 1,\ldots,M-1. \quad \text{[S53]}$$

Therefore, at infinite iterations, I find the asymptotic fraction of cooperators:

$$F_1(N) = M(i)|_{i\to\infty} = R^iM(1) = U\begin{pmatrix} 1 & \ldots & 0 \\ & 0 & \\ \vdots & & 0 \\ & & & \ddots \\ 0 & \ldots & 1 \end{pmatrix}U^{-1}M(1) = R_\infty M(1). \quad \text{[S54]}$$

U is composed of the right eigenvectors of R, $U = \begin{pmatrix} 1 & & 0 \\ 0 & & 0 \\ \ldots & A & \ldots \\ 0 & & 1 \end{pmatrix}$, and can easily show that

$$R_\infty = \begin{pmatrix} z_{00} & z_{01} & & z_{0M} \\ 0 & 0 & & 0 \\ \ldots & \ldots & \ldots & \ldots \\ z_{M0} & z_{M1} & & z_{MM} \end{pmatrix}, \quad \text{[S55]}$$

where z_{ij} are the components of U^{-1}. Therefore, the two vectors $z_N = z_{coop}$, $z_0 = z_{cheat}$ are the two left eigenvectors with eigenvalue 1 of the transition matrix P that satisfy the conditions $z_{00} = 1$, $z_{NN} = 1$, and $z_{Ni} + z_{0i} = 1$.

For the immune cooperator I find $\rho = \frac{n_{i1}}{(n_{i1}+n_{i2})} = \frac{i}{N'}$ as the initial fraction does not change. Therefore, $P_{k\to r} = \binom{N}{r}\left(\frac{k}{N}\right)^r\left(1-\frac{k}{N}\right)^{N-r}$. One can show that in this case, the eigenvalue for the cooperator is $z_{Ni} = i/N$. Therefore, the number of cooperators will be

$$F_I(N) = \left(\frac{1}{2}\right)^N\sum_0^N\frac{i}{N}\binom{N}{i} = 0.5. \quad \text{[S56]}$$

I find that the fraction of immune species will always remain constant in the population under local competition selection. The exact value of $F_1(N)$ for the naive cooperator depends on the details of the competition; however, it is lower bounded by the initial fraction of cooperator-only tubes and will typically be of the same order, $F_I(N) \sim \left(\frac{1}{2}\right)^N$.

6.2. Global competition. n = 1. In global competition, each population is initiated with a *single* strain and grown for a time τ.

Populations are then mixed and plated together. Single lineages are then picked and grown again. All populations are pure, due to the bottleneck of 1. The ratio of pure cooperator population to pure cheater populations increases from cycle to cycle by their relative cell density. If $M(k)$ is the fraction of populations of cooperators, then $\frac{M(k+1)}{1-M(k+1)}=\frac{n_{01}}{n_{12}}\frac{M(k)}{1-M(k)}$. As I assume that cooperators are superior to cheaters, I easily find that $F_g(1) = M(\infty) = 1$. This result is, of course, the case for both the naive and the immune cooperator, as their behavior when unmixed is the same.

***n* = 2.** In this scenario, each population is initiated with two randomly chosen strains and grown for a time τ. Populations are then mixed together. Pairs of cells are chosen randomly from the mix to seed the next round of growth. If r_{coop}, r_{cheat} are the fractions of cooperating and cheating cells found after plating the mixed test tubes, I find the relations

$$r_{coop}(i+1)\propto n_{21}r_{coop}^2(i)+2n_{11}r_{coop}(i)r_{cheat}(i)$$

$$r_{cheat}(i+1)\propto n_{02}r_{cheat}^2(i)+2n_{12}r_{coop}(i)r_{cheat}(i).$$

As $r_{coop}(i) + r_{cheat}(i) = 1$ for all i, I find

$$r_{coop}(i+1)=\frac{n_{21}r_{coop}^2(i)+2n_{11}r_{coop}(i)r_{cheat}(i)}{n_{21}r_{coop}^2(i)+n_{02}r_{cheat}^2(i)+2(n_{12}+n_{11})r_{coop}(i)r_{cheat}(i)}. \quad \textbf{[S57]}$$

The limits of this relation can be found by a fixed-point analysis of the above relation. It is easy to see that the two extremes (r_{coop} = 0, 1) are always fixed points of the above relations. In addition, there would be an intermediate single fixed point, r_{coop}^{st}, if

$$0<r_{coop}^{st}=\left(\frac{2n_{12}-n_{21}}{2n_{11}-n_{02}}+1\right)^{-1}<1. \quad \textbf{[S58]}$$

This will yield a solution between 0 and 1 only if

$$\left(\frac{2n_{11}}{n_{02}}-1\right)\left(\frac{2n_{12}}{n_{21}}-1\right)>0. \quad \textbf{[S59]}$$

The stability of the fixed points can be derived from graphical analysis of the iteration function that governs the recurrence relation, Eq. **S58** (Fig. S4 *C*–*G*),

$$f(s)=\frac{n_{21}s+2n_{11}(1-s)}{n_{21}s^2+n_{02}(1-s)^2+2(n_{12}+n_{11})s(1-s)}s, \quad \textbf{[S60]}$$

and its derivatives at the extremes,

$$f'(0)=\frac{2n_{11}}{n_{02}},\ f'(1)=\frac{2n_{12}}{n_{21}}. \quad \textbf{[S61]}$$

I find that the following conditions will lead to the following fixed points (Fig. S4 *C* and *D*):

a) $f'(1)<1$ and $f'(0)>1$: 100% cooperators
b) $f'(1)>1$ and $f'(0)<1$: 100% cheaters
c) $f'(1)>1$ and $f'(0)>1$: Intermediate stable state
d) $f'(1)<1$ and $f'(0)<1$: Bistable extremes.

What will be these values for the two types of cooperators? For the immune cooperator $\frac{n_{02}}{2}<n_{11}=n_{12}<\frac{n_{21}}{2}$, as I assume that a mix of cooperator and cheater will do better than a cheater only and worse than a cooperator only. This result implies that condition 2 will always hold and the selection will converge to cooperators only. For the naive cheater the conditions met will depend on the exact parameters.

General N. In a similar manner to what I did in the case $n = 2$, I can show that the result of selection with a bottleneck N will depend on the fixed points of the recursion function $f(s)$ and their stability, where

$$f(s)=\frac{\sum_{k=0}^{N}\binom{N}{k}n_{k1}s^k(1-s)^{N-k}}{\sum_{k=0}^{N}\binom{N}{k}(n_{k1}+n_{k2})s^k(1-s)^{N-k}}. \quad \textbf{[S62]}$$

The shape of the recursion function will generally depend on the parameters n_{k1}, n_{k2}. In the following, I generally prove two relations:

a) The immune cooperator has a single stable fixed point for its recursion function, $s = 1$.
b) For the naive cooperator $s = 1$ will always become an unstable fixed point of the recursion function for large enough N.

Proofs of the two relations are as follows:

a) For the immune cooperator, however, this proof has a fairly simple form and I can generally show that for $0 < s < 1$, $f(s) > s$. To this end, I use Eq. **S43** and define

$$n_{tot}(k)=n_{k1}+n_{k2},\ \ n_{k1}=\frac{k}{N}n_{tot}(k). \quad \textbf{[S63]}$$

I can therefore rewrite Eq. **S63** in this case as

$$f(s)=\frac{\sum_{k=0}^{N}\binom{N}{k}\frac{k}{N}n_{tot}(k)s^k(1-s)^{N-k}}{\sum_{k=0}^{N}\binom{N}{k}n_{tot}(k)s^k(1-s)^{N-k}}=\frac{1}{N}\frac{\overline{kn_{tot}(k)}}{\overline{n_{tot}(k)}}>s$$
$$=\sum_{k=0}^{N}\binom{N}{k}\frac{k}{N}s^k(1-s)^{N-k}=\frac{1}{N}\bar{k}. \quad \textbf{[S64]}$$

As $n_{tot}(k)$ is an increasing function of k, I can easily show the relation between the averages:

$$\begin{aligned}\overline{kn_{tot}(k)}-\overline{n_{tot}(k)}\bar{k}&=\overline{(k-\bar{k})\left(n_{tot}(k)-\overline{n_{tot}(k)}\right)}\\&=\overline{(k-\bar{k})(n_{tot}(k)-n_{tot}(\bar{k}))}+\overline{(k-\bar{k})\left(n_{tot}(\bar{k})-\overline{n_{tot}(k)}\right)}\\&=\overline{(k-\bar{k})(n_{tot}(k)-n_{tot}(\bar{k}))}>0.\end{aligned} \quad \textbf{[S65]}$$

The last expression on the right is always positive because of the monotonicity of the function n_{tot}. I have therefore proved that the recursion function $f(s)$ is always larger than s, and this implies that the recurrence relation $s_{i+1} = f(s_i)$ will always have a single limit at $s = 1$: The immune cooperator will always be fixed in the population, irrespective of bottleneck size! Fig. S4 *E* and *F* shows the shape of $f(s)$ for naive and immune cooperators for values of $n = 1, 2, 4, 8, 16$, and 30. From these graphs I can see the striking qualitative difference between the two cooperators. The naive cooperator gains a single stable fixed point between 0 and 1 above a certain bottleneck size. This fixed point approaches 0 as N increases. The immune cooperator, on the other hand, never gains another fixed point, and its only stable fixed point is at $s = 1$. I note, however, that the recursion function of the immune cooperator approaches asymptotically from above the identity function as N increases. This result implies that the convergence time to the fixed point becomes

longer as N increases. In Fig. S4 *E* and *F* I show the numerical value of F_g as a function of N for the two cooperators for 50, 100, and 200 cycles of selection. Clearly, the naive cooperator converges to a specific monotonously decreasing function, whereas the immune cooperator approaches $F_g = 1$, as the number of cycles increases. I note that the growth model is deterministic and assumes an infinite number of demes. An analysis of a more realistic scenario may add other effects of neutral drift.

b) The recursion function $f(s)$ is stable at a fixed point s_f if $f'(s_f) > 1$. $s = 1$ is always a fixed point of the recursion function. I can calculate the derivative of this function at $s = 1$, by expanding it around this fixed point using the variable $y = 1 - s$:

$$f(y) = \frac{\sum_{k=0}^{N}\binom{N}{k}n_c\left(\frac{k}{N}\right)(1-y)^k y^{N-k}}{\sum_{k=0}^{N}\binom{N}{k}n_{tot}\left(\frac{k}{N}\right)(1-y)^k y^{N-k}} = \frac{n_c(1)(1-Ny+o(y^z))+Nn_c\left(1-\frac{1}{N}\right)(y+o(y^z))+y^2\sum_{k=0}^{N=z}\binom{N}{k}n_c\left(\frac{k}{N}\right)(1-y)^k y^{N-k-z}}{n_{tot}(1)(1-Ny+o(y^z))+Nn_{tot}\left(1-\frac{1}{N}\right)(y+o(y^z))+y^2\sum_{k=0}^{N=z}\binom{N}{k}n_{tot}\left(\frac{k}{N}\right)(1-y)^k y^{N-k-z}}$$

$$= \frac{n_c(1)-Ny\left(n_c(1)-n_c\left(1-\frac{1}{N}\right)\right)+o(y^z)}{n_{tot}(1)-Ny\left(n_{tot}(1)-n_{tot}\left(1-\frac{1}{N}\right)\right)+o(y^z)} = 1+Ny\left(n_c\left(1-\frac{1}{N}\right)-n_{tot}\left(1-\frac{1}{N}\right)\right)+o(y^z). \quad \textbf{[S66]}$$

I can therefore use the linear approximation to calculate the derivative of f:

$$\left.\frac{df(s)}{ds}\right|_{s=1} = -\left.\frac{df(s)}{dy}\right|_{y=0} = \frac{N}{n_{tot}(1)}\left(n_{tot}\left(1-\frac{1}{N}\right)-n_c\left(1-\frac{1}{N}\right)\right)$$
$$= \frac{\left(\frac{dn_c}{dx}-\frac{dn_{tot}}{dx}\right)}{n_{tot}}+o\left(\frac{1}{N}\right) = \frac{n_{tot}\frac{dn_c}{dx}-n_c\frac{dn_{tot}}{dx}}{n_{tot}^2}+o\left(\frac{1}{N}\right)$$
$$= \frac{d\left(\frac{n_c}{n_{tot}}\right)}{dx}+o\left(\frac{1}{N}\right) = \frac{df_c}{dx}+o\left(\frac{1}{N}\right) < 1. \quad \textbf{[S67]}$$

I find that for large enough N the fixed point at $s = 1$ becomes unstable.

6.3. Summary. I find that for both local and global selection, the two cooperators behave the same for a bottleneck of a single lineage (as they should, because the cooperators behave identically on their own). However, as bottleneck size increases, the naive cooperator frequency approaches zero, whereas the immune cooperator frequency remains constant (at least after a long period of selection). This striking difference exemplifies the strong selective force for the fixation of the novel cheating immune cooperator.

7. Divergence Is Selected in Complex Models. The model presented in this paper and discussed and analyzed in the previous sections has several simplifying assumptions compared with the realistic scenarios expected for many QS systems:

a) I assume full orthogonality of the different signaling pathways. It is more likely to assume, however, that full orthogonality is the result of gradual evolution through mutations that slowly reduce the crosstalk between pathways.
b) The model does not take into account null alleles in the receptor, the signal, or the public goods enzyme.
c) From a network perspective, the model assumes an open-loop structure, whereas many QS pathways have a positive feedback from an active receptor on both receptor and signal production.
d) The model assumes that the enzyme is a complete public good, but under various conditions the public good may benefit more the producing bacteria, resulting in a snowdrift type of social interaction and not in a prisoner's dilemma type.

In this section I show that divergence is still selected even if any of these simplifications are removed.

7.1. Nonorthogonality of alleles does not prevent their diversification. I use the same Eqs. **S15**–**S18** to define the interaction between various strains, but now assume that each receptor is still activated by the noncorresponding signal but with a lower affinity, $K_{cross}^{ac} = \rho K_{RS}$ with $\rho < 1$. In the following, I show that all selective steps assumed when signals are orthogonal ($\rho = 0$) are kept positive if $0 < \rho < 1$:

Step 1: Selection of a strain with an alternative receptor as a cheater. In this case the new receptor has a lower affinity to the signal. The interaction matrix is therefore $K_I^{ac} = \begin{pmatrix} 1 & 1 \\ \rho & \rho \end{pmatrix}$ and the activation of each receptor is

$$R_1^{active} = \frac{(s_1+s_2)}{K_{RS}+(s_1+s_2)} \quad \textbf{[S68]}$$

$$R_2^{active} = \frac{(s_1+s_2)}{K_{RS}/\rho+(s_1+s_2)}. \quad \textbf{[S69]}$$

Therefore,

$$R_2^{active}-R_1^{active} = \frac{(s_1+s_2)}{K_{RS}/\rho+(s_1+s_2)}-\frac{(s_1+s_2)}{K_{RS}+(s_1+s_2)} < 0 \text{ for } 0<\rho<1. \quad \textbf{[S70]}$$

By using Eq. **S20**, I find that

$$\frac{ds}{dt} = r\frac{P_d}{P_d+1}\left(f\left(R_2^{active}\right)-f\left(R_1^{active}\right)\right)s < 0. \quad \textbf{[S71]}$$

Therefore, strain 2—having the evolved cheater receptor with reduced affinity—will unconditionally invade into strain 1, the original cooperator. I note that the invading "partial" cheater will not lead to a full collapse of the population, as it continues to produce public goods. If final levels of signal are saturating for public goods production, it will have a very similar steady state to the one of the original strain. I note that the lower the reduction in affinity is in the mutant, the slower the invasion dynamics will be, as it is proportional to the difference in public goods production (Eq. **S20**).

Step 2: Immunity of novel cooperator. In this case, a strain with a new signal (R_1S_1, strain 1) evolves from the intermediate cheater strain with the altered receptor (R_2S_1, strain 2). I assume that the novel signal's affinity to the novel receptor is increased back to the original affinity. The interaction matrix now is therefore $K_{\mathrm{II}}^{\mathrm{ac}} = \begin{pmatrix} 1 & \rho \\ 1 & \rho \end{pmatrix}$. Similarly to what is described in *SI Text*, section 5, both strains have the same receptor and therefore

$$f\left(R_1^{\mathrm{active}}\right) = f\left(R_2^{\mathrm{active}}\right) = \frac{(s_1 + s_2\rho)}{K_{RS} + (s_1 + s_2\rho)}. \qquad \textbf{[S72]}$$

Using Eqs. **S10**–**S15** I find that the frequency of the novel cooperator in the system will be maintained:

$$\frac{dn_{\mathrm{tot}}}{dt} = \left(\frac{P_d}{P_{d+1}}\left(1 - rf\left(R_1^{\mathrm{active}}\right)\right) - n_{\mathrm{tot}} - \gamma_n\right)n_{\mathrm{tot}} \qquad \textbf{[S73]}$$

$$\frac{d\left(\frac{n_2}{n_1}\right)}{dt} \propto f\left(R_1^{\mathrm{active}}\right) - f\left(R_2^{\mathrm{active}}\right) = 0 \Rightarrow \frac{n_2}{n_{\mathrm{tot}}} = \alpha \qquad \textbf{[S74]}$$

$$\frac{dS_1}{dt} = \beta_S((1-\alpha)n_{\mathrm{tot}} - S_1) \qquad \textbf{[S75]}$$

$$\frac{dS_2}{dt} = \beta_S(\alpha n_{\mathrm{tot}} - S_2) \qquad \textbf{[S76]}$$

$$\frac{dE}{dt} = f\left(R_1^{\mathrm{active}}\right)n_{\mathrm{tot}} - \beta_E E \qquad \textbf{[S77]}$$

$$\frac{dP_d}{dt} = \mathbf{V}_{\max}E - \beta_{pd}\frac{p_d}{p_{d+1}}n_{\mathrm{tot}}. \qquad \textbf{[S78]}$$

At steady state I easily find $S_1 = (1-\alpha)n_{\mathrm{tot}}$, $S_2 = \alpha n_{\mathrm{tot}}$, and $f(R^{\mathrm{active}}) = \frac{n_{\mathrm{tot}}}{K_{RS}/((1-\alpha)+\rho\alpha)+n_{\mathrm{tot}}}$. This result is equivalent to the steady state of a single strain with public goods production intermediate between those of the cheater strain and the novel immune cooperator strain. As in the case of full orthogonality, any level of population structure in global competition will always lead to the full selection of the novel cooperator.

Step 3: Competition between original and novel cooperators. Note that I did not have to assume anything until now about the affinity of the novel signal to the original receptor. This asymmetry is discussed in *SI Text*, section 8. For the sake of this analysis I assume symmetry, implying that this affinity is also ρK_{RS}. I find that the interaction matrix is therefore $K_{\mathrm{III}}^{\mathrm{ac}} = \begin{pmatrix} 1 & \rho \\ \rho & 1 \end{pmatrix}$. Receptor occupation is therefore:

$$\begin{aligned} R_1^{\mathrm{active}} &= \frac{(s_1 + s_2\rho)}{K_{RS} + (s_1 + s_2\rho)} = g(s+\rho); \\ R_2^{\mathrm{active}} &= \frac{(s_2 + s_1\rho)}{K_{RS} + (s_2 + s_1\rho)} = g(1+s\rho), \end{aligned} \qquad \textbf{[S79]}$$

where

$$g(x) = \frac{x}{K_{RS}/n_2 + x} \text{ and } s = \frac{s_1}{s_2} \approx \frac{n_1}{n_2}. \qquad \textbf{[S80]}$$

g is an increasing function of x and therefore the difference in activation of the two receptors will depend on the value of x. If $n_1 > n_2$, $(s > 1)$, I find that $(1 + s\rho) - (s + \rho) = (\rho - 1)(s - 1) < 0$ and as both $g(x)$ and $f(R)$ are monotonous this result implies (using Eq. **S20**) that if $n_1 > n_2$,

$$\begin{aligned} \frac{ds}{dt} &= r\frac{P_d}{P_d+1}\left(f\left(R_2^{\mathrm{active}}\right) - f\left(R_1^{\mathrm{active}}\right)\right)s \\ &= r\frac{P_d}{P_d+1}(f(g(1+s\rho)) - f(g(s+\rho)))s < 0. \end{aligned} \qquad \textbf{[S81]}$$

Therefore, the levels of the minority strain will always increase and therefore the steady state at $n_1 = n_2$ is stable.

7.2. Divergence in the presence of null mutants. Diversification requires certain types of mutations to the receptor and signal that will occur in a certain order. Most likely this process is a rare event and the majority of signal or receptor mutations will just lead to an effectively null mutant. An important question is whether the presence of these null mutants affects the evolution of diversity. Several points are well worth noting in this context:

a) Although abundant, null mutants are obligate cheaters that are bound to be eliminated by an extreme structured population (such as the very small bottlenecks that often occur in bacterial ecology). The picture emerging from a structured population is one where cooperators are repeatedly invaded by cheaters that are then eliminated and replaced by others. In such a framework two cheater mutants are not required to compete, as they may arise sequentially. Therefore, the intermediate strain R_2S_1 may arise like any other null mutant, on the background of a cooperator strain with negligible interaction with other null mutants. The uniqueness of the intermediate strain is that it can regain cooperation by a second mutation, which cannot be done by a null mutant.

b) Despite these remarks, I carried out a simulation of a structured population three-way competition (*SI Text*, section 6) between the cooperators (either original or immune) and the intermediate strain, in the background of three different types of signaling null mutants: receptor null, signal null, or a double mutant. In addition to the model, I specifically assume that a signaling null mutant is saving the cost of signal, which is defined to be 1/10th of the maximal cost of quorum response. As can be seen in Fig. S5, I find that even under these conditions, the intermediate can invade into the original cooperator strain, but be eliminated in the presence of the immune cooperator. Some peculiarities of the three-way model include a positively selected interaction between the intermediate strain (which makes the signal S_1) and a signal null receptor (which does not make the signal and therefore saves the cost of signaling) whose receptor is induced by S_1. These types of interactions are probably unstable in a more realistic structured population model.

7.3. Generalizing the model to include feedbacks on receptor and signal. I now prove that a generalized system of equations that allow feedback into the production of receptor and signaling molecule and more general cost and benefit terms will show the same behavior of facultative cheating, as long as all generalized functions are monotonously increasing functions and a few other simple rules stand as well. The set of equations is as follows:

$$\frac{dn_i}{dt} = \left(f_1(P)\left(1 - f_2\left([RS]_i\right)\right) - f_3(n_{\mathrm{tot}})\right)n_i \qquad \textbf{[S82]}$$

$$\frac{ds_i}{dt} = f_4\left([RS]_i\right)n_i - \beta_s S_i - n\left(k_+ R_i S_i + k_-[RS]_i\right) \qquad \textbf{[S83]}$$

$$\frac{dR_i}{dt} = fs([RS]_i) - k_+ R_i S_i + k_- [RS]_i - \beta_R R_i \quad \text{[S84]}$$

$$\frac{d[RS]_i}{dt} = k_+ R_i S_i - k_- [RS]_i \quad \text{[S85]}$$

$$\frac{dE}{dt} = P_E \sum_i f_6([RS]_i) n_i - \beta_E E \quad \text{[S86]}$$

$$\frac{dP_d}{dt} = V_{\max} f_7(E) - \beta_{P_d} f_1(P) n_{\text{tot}}. \quad \text{[S87]}$$

I find that time evolution of the ratio $s = \frac{n_1}{n_2}$ is a generalization of Eq. S20:

$$\frac{ds}{dt} = f_1(P)\left(f_2([RS]_2) - f_2([RS]_1)\right)s. \quad \text{[S88]}$$

I now show the following three characteristics of the above system:

i) Cheaters take over cooperators: For a mix of cheaters and cooperators, cheaters' fractions always increase: When considering a QS mutant (strain $i = 1$), I assume that it pays no enzyme production cost, and therefore its cost function is zero. Using Eq. S88 I find that

$$\frac{d(n_1/n_2)}{dt} = \frac{n_1}{n_2} f_1(P)\left(f_2([RS]_2) - f_2(0)\right) > 0. \quad \text{[S89]}$$

The inequality is true because f_2 is monotonously increasing.

ii) Divergent QS systems are facultative cheaters with $n_1 = n_2$ as a stable steady state. I want to show that if $n_1 < n_2$ then $\frac{d(n_1/n_2)}{dt} > 0$, and vice versa. To show this result I want to show that

$$n_1 < n_2 \Rightarrow f_2([RS]_2) > f_2([RS]_1) \Leftrightarrow [RS]_2 > [RS]_1, \quad \text{[S90]}$$

where the equivalence stems from the monotonous increasing property of f_2. If I assume that the change in cell density is slow compared with signaling molecule level, receptor–signaling molecule binding, and receptor production, I can assume a quasi-steady state for Eqs. S82–S87 and find

$$\frac{d[RS]_i}{dt} = g(n_i, [RS]_i) = n_i \frac{f_4([RS]i)}{\beta_s K_{RS}} f_5([RS]_i) - \beta_R [RS]_i. \quad \text{[S91]}$$

The quasi-steady state solution of Eq. S91 is given by $S_i = K_{RS}\beta_R \frac{[RS]_i}{f_5([RS]_i)}$. The condition for $[RS]_i$ to be monotonous with S_i is {I define $s([RS]_i) = f_4([RS]_i)f_5([RS]_i)$}

$$\frac{d[RS]_i}{dn_i} = \frac{1}{dn_i/d[RS]_i} = \frac{s([RS]_i)^2}{s([RS]_i) - [RS]_i s'([RS]_i)} > 0 \quad \text{[S92]}$$

or

$$s([RS]_i) > [RS]_i\, s'([RS]_i). \quad \text{[S93]}$$

Eq. S77 is also the condition for the stability of the quasi–steady-state solution:

$$\left.\frac{dg}{d[RS]_i}\right|_{g=0} < 0 \Leftrightarrow \frac{n_i}{K_{RS}} s'([RS]_i) - \beta_R < 0 \Leftrightarrow [RS]_i s'([RS]_i) < s([RS]_i). \quad \text{[S94]}$$

Therefore, every stable steady state of receptor level follows a monotonous increasing dependence on cell density, as required by Eq. S92.

Note that I assume here that all reactions are faster than the change in cell density for the quasi–steady-state approximations to be valid. Nevertheless, the conditions in Eq. S92 will be true under more general parameters.

iii) Cooperation is beneficial: A community of cooperators will have a higher cell density than a community of cheaters. Using Eqs. S82–S87 I find that the density of pure cooperators and cheaters is

$$n_{\text{coop}} f_3(n_{\text{coop}}) = \frac{V_{\max}}{\beta_{P_d}} f_7(E)\left(1 - f_2([RS]_i)\right) \quad \text{[S95]}$$

$$n_{\text{cheat}} f_3(n_{\text{cheat}}) = \frac{V_{\max}}{\beta_{P_d}} f_7(0). \quad \text{[S96]}$$

Therefore, the condition for $n_{\text{coop}} > n_{\text{cheat}}$ (as f_3 is monotonously increasing) is

$$f_7(E)\left(1 - f_2([RS]_i)\right) > f_7(0). \quad \text{[S97]}$$

This condition basically reflects the condition that benefit of cooperation will be larger than the cost of cooperation.

7.4. Diversification of quorum sensing under snowdrift conditions. In this section I demonstrate that QS systems can diversify also under conditions where the quorum response leads to a snowdrift type of social interaction between the producing and nonproducing strains. The snowdrift game between two players occurs when the payoff matrices imply that defection by one player will not lead to the defection of the other player. In a multiplayer system (like the case of bacteria), snowdrift conditions will lead to coexistence of producers (cooperators) and nonproducers (cheaters). Recently, Gore et al. demonstrated that sucrose metabolism by invertase secretion in yeast leads to coexistence of producers and cheaters, interpreted as a snowdrift game (21). They demonstrated that partial localization of the enzyme to the yeast periplasm leads to internalization of a small fraction of the glucose produced by sucrose cleavage directly into the enzyme-producing cell. They showed that preferential growth at a low glucose level and the preferential internalization of glucose by the producing cells can lead to the observed snowdrift game.

Following Gore et al. (21), I model snowdrift conditions by assuming that the enzyme is bound to the producing cell and that a fraction ε of the substrate is directly internalized by the producing cell. I also assume that the growth rate of each cell is proportional to the availability of nutrient to the power α. I therefore need to write a new set of equations where each strain has also an enzyme population, E_i, and a local nutrient pool, P_i. The set of equations defining the population dynamics now is therefore

$$\frac{dn_i}{dt} = \left(H(P_i + P_{\text{pub}}, \alpha)\left(1 - rf(R_i^{\text{active}}) - r_s\right) - n_{\text{tot}} - \gamma_n\right)n_i \quad \text{[S98]}$$

$$\frac{ds_i}{dt} = \beta_s(n_i - S_i) \quad \text{[S99]}$$

$$\frac{dE_i}{dt} = f\left(R_i^{\text{active}}\right)n_i - \beta_E E_i \qquad \textbf{[S100]}$$

$$\frac{dP_i}{dt} = \varepsilon V_{\max} E_i - \beta_{P_d}\left[\frac{P_i}{P_i + P_{\text{pub}}} H\left(P_i + P_{\text{pub}}, \alpha\right)\left(1 - rf\left(R_i^{\text{active}}\right) - r_s\right)\right] \qquad \textbf{[S101]}$$

$$\frac{dP_{\text{pub}}}{dt} = \left(J_{P_d} + \sum_i \left[(1-\varepsilon)V_{\max} E_i - \beta_{p_d}\frac{P_{\text{pub}}}{P_i + P_{\text{pub}}} H\left(P_i + P_{\text{pub}}, \alpha\right) \times \left(1 - rf\left(R_1^{\text{active}}\right) - r_s\right)\right] n_i\right) \Big/ n_{\text{tot}}. \qquad \textbf{[S102]}$$

Unlike in Eqs. **S15**–**S18**, I have defined the enzyme and nutrient pools here for a single bacterium, because of the private nature of some of the nutrients. R_i^{active} is defined as in Eq. **S14** for the different pairs of strains. The function H is the Hill function:

$$H(x, \alpha) = \frac{x^\alpha}{1 + x^\alpha}. \qquad \textbf{[S103]}$$

Note that when $\varepsilon = 0$ and $\alpha = 1$, the above equations are essentially equivalent to Eqs. **S15**–**S18**.

In Fig. S6 I show the results of competition between the different divergent strains under homogenous conditions (Fig. S6 *A–D*) and in a structured population model identical to the one discussed in *SI Text*, section 6 (Fig. S6 *E–H*). The only difference in the homogenous conditions is that the cheater strain R_2S_1 invades into the original QS strain R_1S_1 only to coexistence of ~75% (the number depends on the exact parameters used). As in the prisoner's dilemma case, the fraction of the novel immune cooperator, R_2S_2, does not change with time in its competition with R_2S_1, so the immunity property remains the same. This result is clear, as immunity is related to the nature of signaling and not to the nature of social competition. Finally, the competition between the strains R_2S_2 and R_1S_1 has the 50%:50% coexistence state, as an only stable state, whereas the cheater: cooperator coexistence frequencies are destabilized.

Fig. S6 *E–G* demonstrates the fate of the three competitions between the strains under a structured population with global competition (as in Fig. 3 of the main text). As you can see, the novel strain R_2S_2 is selected under these conditions. Because snowdrift conditions lead to coexistence of the cheater R_2S_1 and the original cooperator R_1S_1, I considered also the three-way competition between R_1S_1, R_2S_1, and R_2S_2. As shown in Fig. S6*H*, the two cooperators reach coexistence under these conditions whereas the intermediate strain is eliminated.

In summary, the snowdrift nature of the social interactions does not prohibit the evolution of diversified quorum-sensing pathways.

8. The Evolution of Cross-Interactions. When considering the observed relations between various strains carrying divergent forms of the same QS system, one can find several types of cross-interactions; in more closely related QS systems (from a sequence perspective), one often finds some level of cross-activation that may not be necessarily equal between different strains. In more distantly related QS systems, one often finds cross-inhibition between the two strains, again, not always to the same extent. Both cross-inhibition and cross-activation may be a direct effect of mechanistic inability to fully diverge or result from specific adaptations that select for those interactions. In this section I consider the second option, to understand the full richness of social interactions arising during diversification.

8.1. Cross-inhibition is maintained in a complex population structure. As explained in the main text, cross-inhibition is a facultative cheating strategy of the inhibited strain in my model: It will produce public goods when alone and avoid producing them when in the presence of the inhibiting strain. I claimed that this strategy cannot be eliminated well by a structured population as demonstrated by the analysis of random bottlenecked populations.

This argument can be understood by following the steady-state levels of the three strains for the three-way competition and the three two-way and three one-way competitions, as presented in Fig. S7*A*. As can be seen, in the three-way competition cross-inhibition is strongly selected over orthogonality. In two-way competition cross-inhibition will be selected over orthogonality if the inhibited orange strain performs better than the orthogonal orange strain when in competition with the cyan strain—that is, if $\Delta_1 > 0$. Otherwise, a two-way competition will select for orthogonality. Also, the two-way competitions leads to cooperation between the two orange strains (orthogonal and inhibited) against the cyan strain—if $\Delta_1 > 0$, then the only strain that loses in the two-way competition is the cyan strain. One-way growth is always equivalent for all strains. Different structured populations will assign different probabilities for the different types of competitions. In the random bottleneck model with bottleneck size N, the total number of initial conditions is 3^N, of which only $\sim 3 \times 2^N$ cases are of two-way or one-way type (where one or more of the strains are absent from initial conditions). Therefore, over $N \cong 5$ the three-way competition will dominate the spectrum of competitions and cross-inhibition will evolve. For $n < 6$ I may find a parameter-specific solution to the problem.

The evolution of one-sided cross-inhibition can lead to the evolution of mutual cross-inhibition, as a cheating strategy of the other (cyan) strain.

8.2. Unilateral cross-activation can be a direct outcome of divergence and benefit the signaling strain. In the model presented in this paper I assumed that one system with receptor R_1 and signaling molecule S_1 will diverge into a novel QS system with receptor R_2 and signaling molecule S_2. I assumed that R_2 and S_2 are specific and do not interact with the original strains. A closer observation of the evolutionary dynamics exposes further complexity: The second signal, S_2, is actually not selected against activation of R_1. This asymmetry between S_1 and S_2 is formed because the mutations leading to the formation of S_2 occur in the strain R_2S_1 and are selected to activate R_2. Because R_1 is already absent at this stage, there are no selective constraints on its interaction with S_2.

What are the implications of such unilateral cross-activation: S_2 activating R_1, but S_1 not activating R_2? In Fig. S7*B* I demonstrate the striking difference between the case of a pair of non-interacting divergent strains and a pair with unilateral cross-activation. In the former case, analyzed above and in the main text, the two strains will coexist with equal densities. In the latter case, the signaling cell will become dominant once the strength of cross-activation (the affinity of S_2 to R_1) becomes of the same order of magnitude as the self-affinity of the two systems. This result occurs because now the signaling QS strain is inducing the original QS strain to work also under conditions where it has not reached a quorum. Therefore, a possible scenario for selection for divergence is a nonsymmetric relation with the evolved strain dominating over the previous strain.

What are the possible adaptations the cross-activated QS strain can undergo to counteract a cross-activation? There are several possible answers with different functional outcomes:

i) Receptor divergence: The receptor can further diverge to lose the cross-activation. This action will return the system to the scenario described in the main text.

ii) Inhibition: The receptor can diverge to be inhibited by the signal, as was discussed in the previous subsection. It is not

clear a priori whether option *i* or *ii* is more accessible to the receptor.

iii) Overactivation: In this case, the signal of the induced strain will mutate to induce the receptor of the inducer strain. The cross-induction is described in *SI Text*, section 3. It effectively reduces the difference between the strains and in general reduces the chances of mutual invasion. It is unlikely that this type of mutation will be selected under the inducing selective pressure, because it does not benefit the mutant under these conditions (it will survive, but not increase in frequency).

9. Comparison Between QS Divergence and Kin-Recognition Systems. In the last decade, kin discrimination has gained a considerable interest from evolutionary biologists and sociobiologists and several models analyzing the social dynamics of kin-recognizing cooperators have been devised (22–26). All of these models share several assumptions: First, they assume that kin discrimination is characterized by a single locus (tag locus) with multiple alleles (tag colors). Second, another locus (cooperation) has two alleles, cooperate or defect. Third, they assume kin-specific cooperation: Cooperators will cooperate only with organisms with the same tag color, whereas defectors will always defect. The underlying question of all of the works is whether kin recognition can ease the evolution of cooperation by directing cooperative behavior only toward kin with higher relatedness than the average (26) (Fig. S8 *A* and *B*). Note that unlike a greenbeard locus, kin recognition is a multilocus system and is not supposed to be immune from defection (or cheating)—an organism carrying a specific kin phenotype can still be a defector.

The main findings of these works (as this author understands them) are that kin recognition can evolve and benefit cooperation only if population structure is complex enough (24, 26) or linkage disequilibrium between the kin and cooperation loci is high (23) or if the kin locus mutation rate is very high compared with the defection mutation rate (25). In the generic case (and specifically in well-mixed environments with relatively low mutation rates), the population will consist of a single kin-recognition type, making the allele ineffective in promoting cooperation. This kin-recognition allele will occasionally mutate into a new allele; if this mutation happens in a cooperator and the general level of cooperation of the previous kin type is low, then it will quickly invade to fixation (23, 24). The underlying reason for this behavior is the positive frequency selection that is imposed by kin recognition: The majority kin is more likely to meet counterparts (which by lineage are likely first to be cooperators) and gain benefits, compared with a minority kin that only rarely meet its own kin.

The model I present for the evolution of QS is different from all of the above models in two critical aspects:

i) Two loci for kin type: All of the previous models assumed the existence of a single locus for kin type. My model has the more realistic assumption that at least two loci are needed for kin recognition: a locus for tag display and a locus for tag recognition (Fig. S8*C*). This problem was mentioned briefly in one of the above works (23) and it was suggested that neutral evolution can lead to a coordinated switch in both loci. My model, to the contrary, shows that coevolution of tag and recognition loci is positively selected through cheating and immunity. Whereas my model assumed a public signal and a public good, this result can be shown to be true also in a model of direct cooperation between pairs, for exactly the same reasons (Fig. S8*D*).

ii) Negative frequency selection on alternative kin types: In contrast to the previous models, my model results in coexistence of different kin types even in homogenous conditions. This fundamental difference between my model and the rest of the models stems from the public goods nature of cooperation: In my model, organisms decide to cooperate on the basis of the presence of kin, but once cooperating, they will benefit all of the surrounding organisms, not just their kin. This non-selective cooperation stabilizes minority strains that invade through facultative cheating dynamics but comes with an obvious cost: Public goods cooperation through kin recognitions cannot increase the level of cooperation in a population (and may decrease it). This cost is clearly so, as defectors (irrespective of their kin type) will always exploit active cooperators.

iii) Whereas the kin recognition system described in my model does not lead to an increase in the total level of sociality, it does serve to diversify the number of "colors" used in the recognition system. The focus of my work is to explain this pattern of diversity and not to suggest new mechanisms for stabilization of cooperation.

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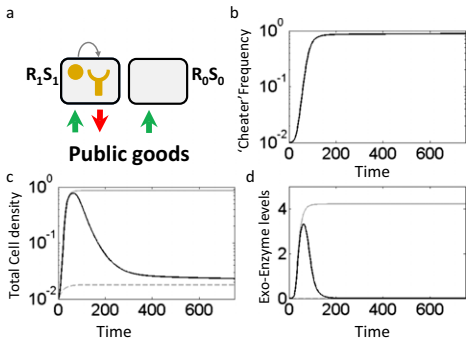

Fig. S1. QS^- is a cheater. (*A*) A QS^- strain (R_0S_0, cheater) can invade a QS^+ strain (R_1S_1, cooperator) by not paying the cost of public goods production (red arrow), but gaining its benefits (green arrow). (*B–D*) Numerical results of an invasion of a QS^- strain into a QS^+ strain. I use Eqs. S15–S18 with $K_{ij}^{ac} = \begin{pmatrix} 0 & 0 \\ 0 & 1 \end{pmatrix}$. Initial QS^- (strain 1) frequency is set to 1% of the producer frequency. Shown are the following: (*B*) Frequency of the QS^- strain. This frequency increases with time. (*C*) Total cell density. This density initially increases, but as the cheater strain invades the population, it decreases again. Total cell densities of a pure QS^+ population (gray line) and QS^- population (dashed gray line) are also shown. (*D*) Public goods (enzyme) levels. These levels follow a similar trend to that of total cell density. Gray lines show enzyme production in QS^+ only and QS^- only strains.

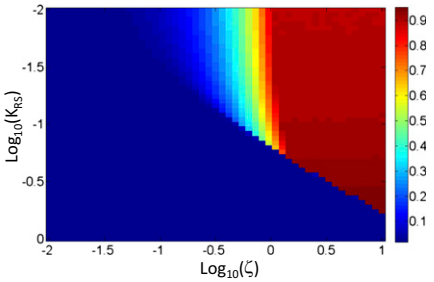

Fig. S2. The steady-state value of cooperator for different levels of ζ, K_{RS}.

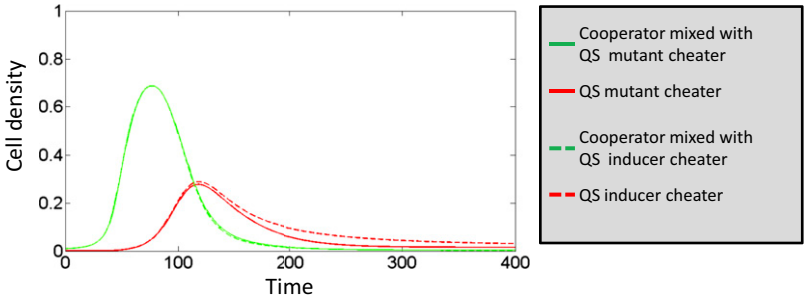

Fig. S3. Properties of an inducer cheater. Shown are the cell densities as a function of time of a quorum-sensing strain (green) that is mixed with a cheater (red) who has a 100-fold smaller initial cell density. Two types of cheaters are compared: complete QS null cheater (solid lines) that lacks both receptor and signal and an inducer cheater that is still producing the signal and therefore induces the cooperator strain's quorum response. As can be seen, in my model, the inducer cheater levels are maintained higher for a longer time. Nevertheless, the qualitative behavior of the cheater is independent of induction.

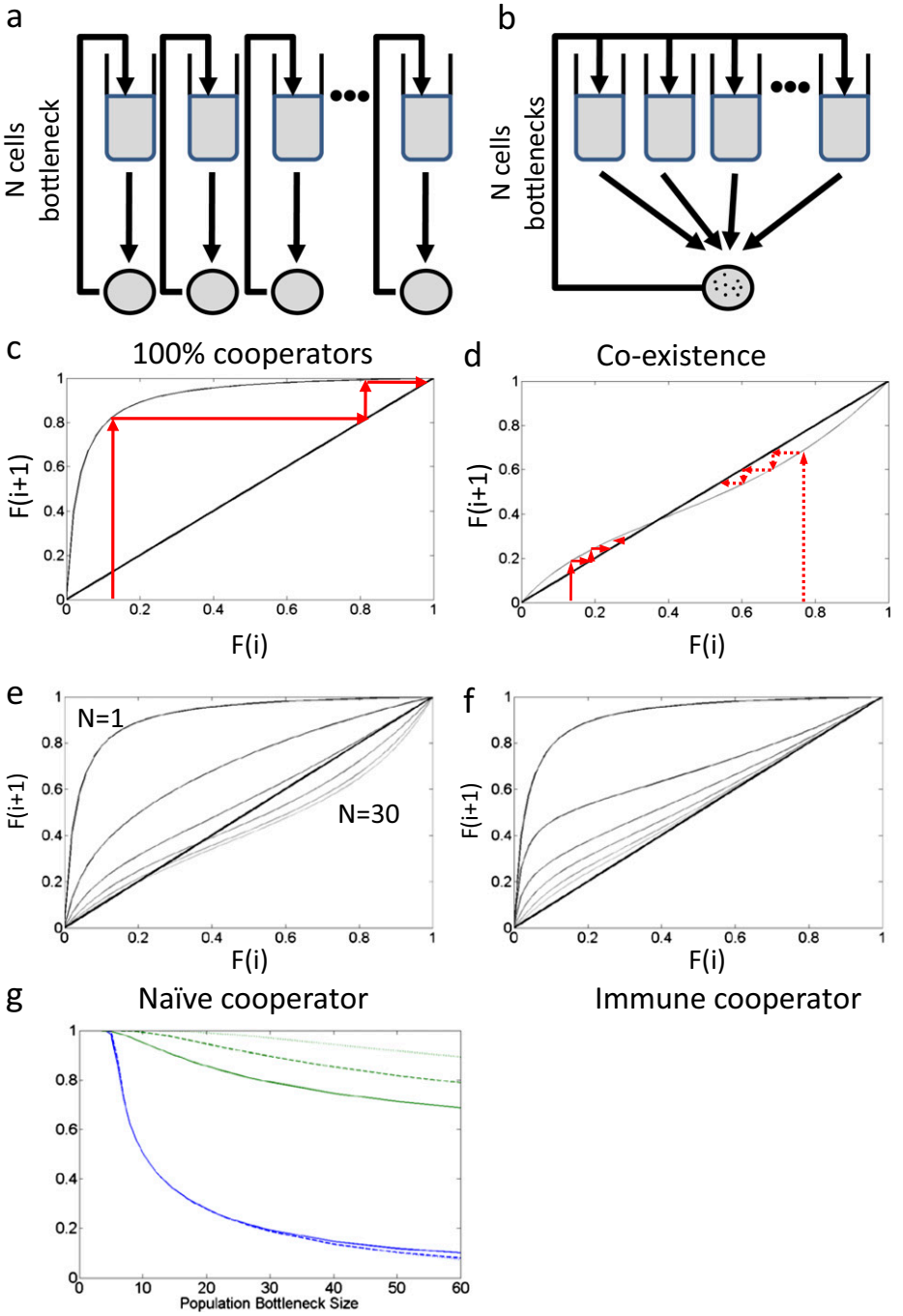

Fig. S4. Selection in a structured population based on bottlenecks. (*A* and *B*) The two selection processes. (*A*) Local competition. (*B*) Global competition. (*C*–*G*) Cooperator frequency in global competition can be calculated using iteration maps. (*C* and *D*) The frequency of cooperators after a cycle is a function of their frequency before the cycle. The limit of many cycles can be calculated from the iteration map as shown in (*C*) the 100% cooperator map and (*D*) coexistence of cooperators and cheaters. (*E* and *F*) Iterations map for (*E*) naive cooperator and (*F*) immune cooperator for varying bottleneck size. Shown are the maps for bottleneck size $N = 1, 2, 4, 8, 16$, and 30. $N = 1$ and 30 are indicated in *E* and the colors are the same for both plots. As can be seen, the naive cooperator's map has a coexistence fixed point for $n > 4$. The immune cooperator always has only the pure cooperator as a fixed point, but as N increases the iteration map convergence to the identity function and convergence time to pure cooperators become longer. (*G*) Cooperator frequency as a function of bottleneck size for the naive (blue) and immune (green) cooperators for 50 (solid line), 100 (dashed line), and 200 (dotted line) selection cycles. As can be seen, the naive cooperator's dependence converges quickly to a limit, whereas the immune cooperator's dependence converges slowly toward pure cooperators.

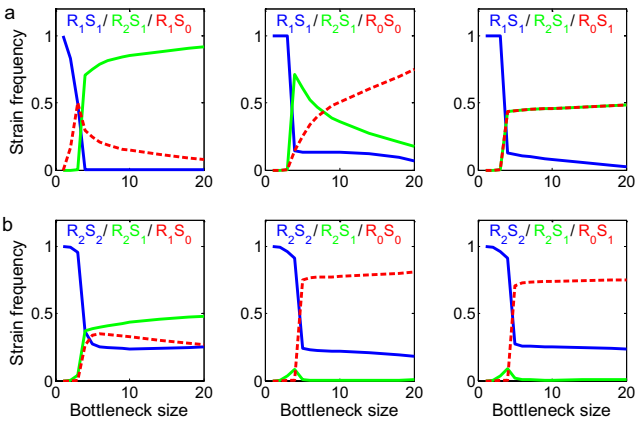

Fig. S5. The effect of obligate cheaters on the diversification of QS systems. (*A* and *B*) Shown are the frequencies of the various competing strains as a function of bottleneck size in a structured population three-way competition. The competing strains are the cooperator [blue, either the naive (*A*) or the immune (*B*)], the intermediate strain R_2S_1 (green), and one of three types of null mutants of the original naive cooperator R_1S_1 (red). These types are signal null mutant (*Left*), signal and receptor null mutant (*Center*), and receptor null mutant (*Right*). I assume here that constitutive signaling has a constitutive cost that is saved by the signal null mutant. This cost is 10 times lower than the maximal quorum response cost. As can be seen, the intermediate mutant strain R_2S_1 always invades into the original cooperator R_1S_1 and is invaded by the immune cooperator R_2S_2. R_2S_1 is not eliminated by the immune cooperator when in the presence of a signaling mutant of the original cooperator. This outcome is because the signal of R_2S_1 is inducing a quorum response from the null mutant. I note here that the exact fate of the three strains will strongly depend on the length of growth and the assumptions about the structured population. A three-way Poisson distribution as I use here (*SI Text*, section 6) is probably unrealistic. A more likely scenario is that most demes carry only two of the three strains and that three-way competition is rare. Parameters of the model are the same as defined in *Materials and Methods* in the main text. Signaling mutants are assumed to save a constitutive cost that amounts to $r_s = \frac{r}{10} = 0.01$ of the maximal growth rate. Interaction matrices are defined appropriately to reflect the various types of strains.

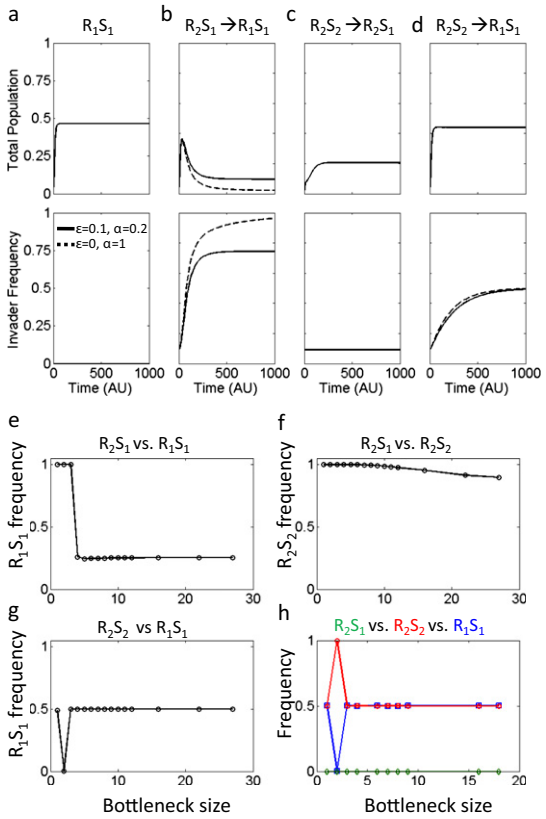

Fig. S6. The selection of QS diversification in a snowdrift interaction model. (*A–D*) For each case I show the total population as a function of time (*Upper*) and the frequency of the invading strain as a function of time (*Lower*). For each situation I show the result of a snowdrift type of interaction (solid line, $\varepsilon = 0.1$, $\alpha = 0.2$) and a prisoner's dilemma type of interaction (dashed line, $\varepsilon = 0$, $\alpha = 1$). The cases are (*A*) a single cooperator strain, R_1S_1 [no competition and hence no invader (*Lower*)], (*B*) R_2S_1 invades into R_1S_1. Note that in the snowdrift case the cheater strain invades only to a frequency of ~75%. (*C*) R_2S_2 invades into R_2S_1. The immune cooperator is neutral in invasion—frequency remains constant with time. (*D*) R_2S_2 invades into R_1S_1. The two strains coexist in equal frequency. (*E–H*) Competition in a snowdrift model under a structured population. (*E–G*) Shown are the results of global competition under a structured population as a function of bottleneck size. The competitors are shown at the top, and the *y* axis defines which strain's frequency is shown (the other strain's frequency complements to 1). The results are essentially the same as for a prisoner's dilemma case: The intermediate strain invades for a large enough bottleneck size (*E*), whereas the immune cooperator fully invades the intermediate strain (*F*). The two competing quorum-sensing strains coexist except for the case of a bottleneck size of 2, where the system is bistable. (*H*) As the intermediate strain R_2S_1 coexists with the original quorum-sensing strain R_1S_1, I must consider also the triple competition between R_1S_1 (blue), R_2S_1 (green), and R_2S_2 (red) to determine the fate of strain R_2S_2 after its formation. Shown are the frequencies of the three strains as a function of bottleneck size. For all cases (except for a bottleneck of size 2), the two divergent strains coexist in a frequency of 50% whereas the intermediate strain is eliminated.

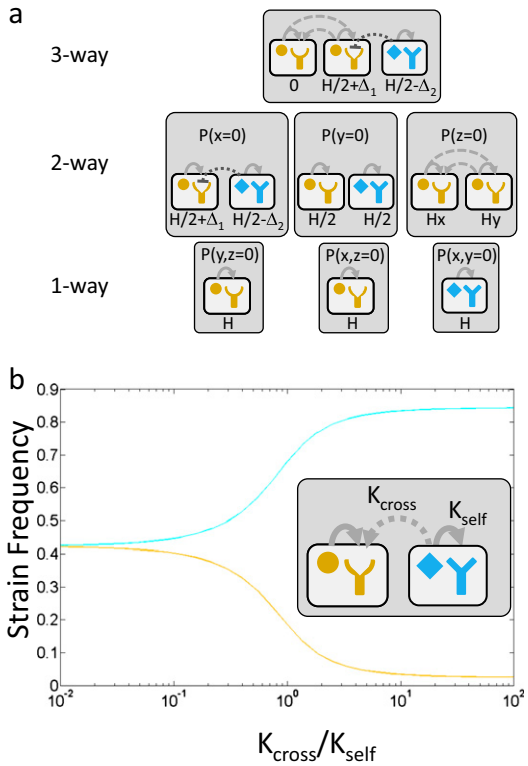

Fig. S7. The effects of cross-interaction between divergent strains. (*A*) Cross-inhibition is a cheating strategy of the receiving strain. Schemes show the interaction between the communication systems of three strains: two divergent ones (orange and cyan) and a mutant of the orange system in which the receptor is inhibited by the signal of the cyan strain. Shown are the interactions during a three-way competition as well as those for two-way and one-way populations. The expression below each term defines the approximate steady-state cell density of this strain in the specific competition. The expression on the top of each competition box displays the probability of attaining this competition. *x*, *y*, and *z* are the initial frequencies of the orthogonal orange, inhibited orange, and cyan strains, respectively. However, three-way competition always leads to the elimination of the orange orthogonal strain (Fig. 3 *B* and *C*). The results of two-way competitions are parameter dependent. The three strains are equivalent during one-way growth. (*B*) Cross-activation is a cheating strategy of the signaling strain. If signal from one strain (cyan in this case, see scheme) cross-activates the receptor of the second strain, but not vice versa, the cross-activating strain will cheat on the activated strain, by inducing its public goods production at lower concentrations. This result is similar to the case of an inducer–cheater discussed in *SI Text*, section 4. The graph shows the steady-state levels of the two strains for varying levels of the ratio between cross-activation affinity and self-activation affinity.

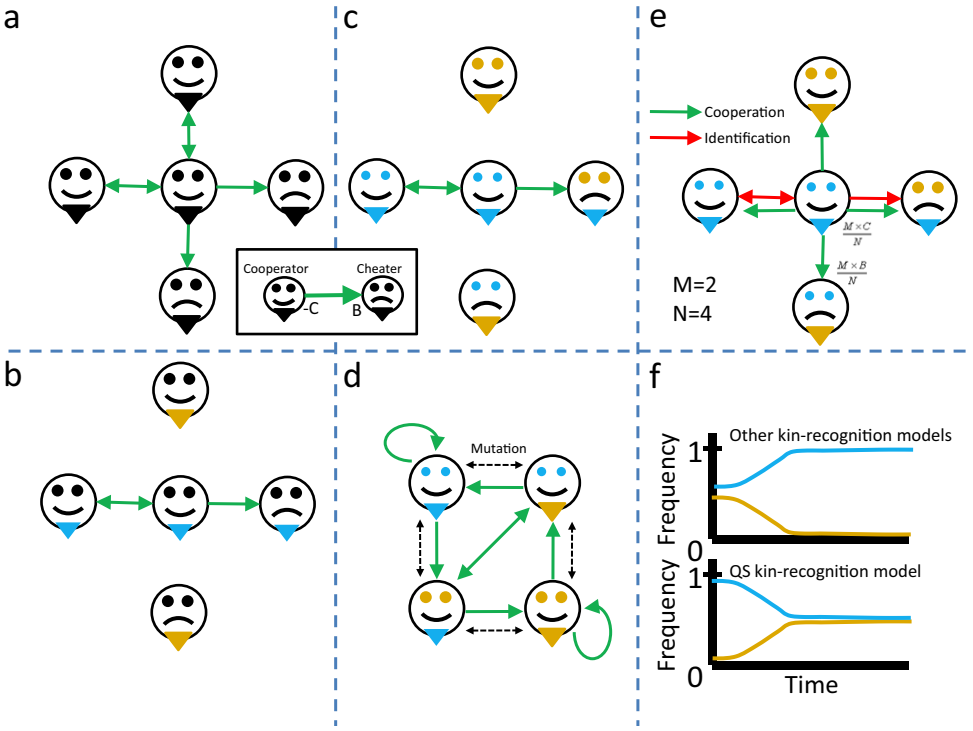

Fig. S8. Kin recognition and QS diversification model. (*A*) Typical models of cooperation include two types of strategies: cooperator (*Inset*, smiley face) and cheater (*Inset*, sad face). A cooperator will invest a cost *C* to give its partner (either a cooperator or a cheater) a benefit *B*. A green arrow directs from cooperator to beneficiary. (*B*) Kin-recognition models assume that each organism is characterized by a tag (orange or cyan beard in this case). A cooperator will invest and benefit only an organism with the same tag. (*C*–*E*) The QS model differs in two substantial ways from previous kin recognition models. (*C* and *D*) Two-locus kin recognition. (*C*) The QS diversification model applies to a case where kin is represented by two loci: a recognition locus (eye color) and a tag locus (beard color). A cooperator will invest and benefit only when it recognizes a tag of the same color, i.e., the cooperator's eyes should have the same color as the beneficiary's beard. (*D*) The social and genetic relations between the four types of cooperators for two alleles/two loci kin types. Black dashed arrows represent mutations. Blue arrows represent social interaction. Social relations form a cycle of social helping between the four genotypes. Unicolored organisms help themselves whereas multicolored organisms help each other. Each of the organisms can also mutate into a cheater that can cheat its ancestor and the cooperator preceding it in the cycle. (*E*) Decoupling help and recognition. The QS model assumes that identification (red arrows) leads to cooperation (green arrows), which equally benefits all neighbors of the cooperators (including nonkin). For the example above, the number of identified neighbors is $M = 2$, leading to a cost of investment $M \times C$ for the cooperating cell and a benefit $\frac{M \times C}{N}$ for each neighbor ($n = 4$ in this case). (*F*) Frequency-dependent selection in the two models. (*Upper*) In other kin recognition models, the selection is positive frequency dependent—the majority strain will be fixed in the population. (*Lower*) Decoupling between cooperation and identification cannot promote the level of cooperation but leads to negative frequency selection and coexistence of multiple kin types.

Table S1. Examples of secreted QS-dependent molecules in various bacteria, including known species with a divergent quorum-sensing system

Organism	Gram +/–	QS signaling gene	Signal type	QS-regulated secreted molecule	Function	Ref.	Notes
Bacillus subtilis	+	*comX*	Modified peptide	Surfactin (srf)	Surfactant	(1)	Diverging signal
Staphylococcus aureus	+	*agr*	Modified peptide	Alpha toxin (hla)	Hemolysin		Diverging signal, cross-inhibition
Streptococcus pneumoniae	+	*comC*	Unmodified peptide	*iga*	IgA1 protease	(2)	Diverging signal
Bacillus cereus	+	*papR*	Unmodified peptide	Hemolysin	Hemolysin	(3)	Diverging signal
Erwinia carotovora	–	*expI*	AHL (3-oxo-C6-HSL/3-oxo-C8-HSL)	*Cel*	Cellulase	(4)	Diverging signal
Chromobacterium violaceum	–	*cviI*	AHL (C6-HSL/3-oxo-C10-HSL)		Chitinase	(5–7)	Diversifying signals, cross-inhibition
Pseudomonas aeruginosa	–	*lasI*	AHL	Elastase	Protease	(8)	
P. aeruginosa	–	*rhlI*	AHL	Rhamnolipid	Surfactant	(8)	
Ralstonia solanacearum	–	*solI*	AHL	Egl	Endoglucanase	(9)	
Serratia proteamaculans	–	*sprI*	AHL	*lipB*	Secretion machinery for multiple exo-enzymes	(10)	
Burkholderia cenocepacia	–	*cepI*	AHL	zmpA	Extracellular zinc metalloprotease	(11)	
Lactococcus lactis	+	*nisA*	Nisin	Nisin	Antibiotic	(12)	The antibiotic is also the signal
Enterococcus faecalis	+	*fsrB*	Modified peptide	Gelatinase	Extracellular zinc metalloprotease	(12)	

AHL, acyl homoserine lactone.

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