5 | ON THE EVOLUTIONARY ORIGIN OF QUORUM SENSING AS A MECHANISM FOR COLLECTIVE SENSING

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ABSTRACT

Bacteria release and sense small molecules called autoinducers (AI) in a process known as 'quorum sensing' (QS). The classical interpretation of QS states that bacteria, by means of sensing the AI concentration, estimate the population density in order to regulate the expression of functions that are only beneficial when carried out by a large number of cells. A major challenge to this interpretation is that the AI concentration strongly depends on the environment, rendering AI-based estimates of cell density unreliable. Here we propose an alternative interpretation of QS, where bacteria, by releasing and sensing AI, use social interactions to improve their individual estimate of the environmental conditions (i.e., benefit from 'the wisdom of the crowd'). Using an evolutionary model, we show that this benefit alone can lead to the evolution of QS and reconcile the observed dependency of QS on both cell density and the environment. Additionally, it can explain why QS regulates many traits that offer a fitness advantage regardless of the behavior of neighboring cells.

5.1 INTRODUCTION

Many traits of major relevance for bacteria are controlled by quorum sensing (QS). QS is a process where bacteria synthesize small molecules known as autoinducers (AIs) that are either passively or actively released into the extracellular space. The same bacteria that can produce AIs also sense and respond to the extracellular concentration of AIs by using specialized receptors that bind these molecules and initiate signal transduction cascades once the AI concentration is above a certain threshold. These signaling cascades have been well described in many systems and regulate processes like biofilm formation, virulence, competence and sporulation (Fuqua et al., 1994; Miller & Bassler, 2001; Waters & Bassler, 2005). Despite the detailed understanding of the molecular mechanisms underlying various QS systems, the adaptive value and evolutionary origin of QS are less understood (Lerat & Moran, 2004; West et al., 2012). The classical functional interpretation of QS states that bacteria engage in releasing and sensing AIs in order to monitor population density. By regulating the expression of a trait based on cell density, bacteria would ensure that this trait is expressed only when a sufficiently high number of other cells (hence the term 'quorum') are also expressing it (Fuqua et al., 1994; Waters & Bassler, 2005).

This interpretation is based on two premises that have been challenged in light of accumulating evidence on the diversity and complexity of QS systems. The first is that the benefit of expressing a QS-regulated trait for an individual increases with population density. There is evidence that this premise holds for QS systems that regulate traits involving the production of 'public goods' (e.g., extracellular proteases), since the benefit of expressing such traits increases if more cells also express them (Darch, West, Winzer, & Diggle, 2012). However, QS also regulates the expression of private functions, such as metabolic enzymes or competence, that are not shared with other cells and thus provide density-independent benefits (Schuster, Sexton, and Hense, 2017; Darch et al., 2012). Hence, it is unclear why bacteria would regulate these functions by monitoring cell density.

The second premise is that bacteria can reliably estimate population density and the potential for fitness gains by sensing the local AI concentration. This assumption has been notably challenged by studies in different QS systems demonstrating that the relationship between cell density and the concentration of AI can be highly contingent on environmental conditions. The best-known environmental factor mediating this relationship is the diffusivity of the extracellular environment. For instance, at sufficiently low diffusivity the AI concentration can be such that the quorum for QS induction is a single cell (Carnes et al., 2010). Such prominent role of environmental diffusivity on the AI concentration led to the 'diffusion sensing' hypothesis. This hypothesis states that bacteria release AI to test the diffusivity of the medium and regulate accordingly the secretion of costly molecules to the extracellular environment (Redfield, 2002). However, given that many other factors such as pH, oxygen and antibiotic stress can affect QS systems (Decho, Norman, & Visscher, 2010; Horswill, Stoodley, Stewart, & Parsek, 2007; Moreno-Gámez et al., 2017), emphasizing diffusion as the main functional driver of QS likely underplays the complexity of QS

regulation. An alternative, more integrative perspective acknowledges that multiple biotic and abiotic factors regulate QS and that responding to a combination of these factors rather than to a single one of them better explains the functional role of QS for bacteria in nature (Cornforth et al., 2014; Moreno-Gámez et al., 2017; West et al., 2012).

If QS is indeed a mechanism to respond to the environment, this raises the question of how bacteria evolve to employ direct and/or collective sensing of environmental information. Here we propose that bacteria benefit from regulating gene expression through QS because cell-to-cell communication allows individual cells to more accurately determine the state of the environment at spatially relevant scales, which in turn enables individual cells to make a more informed decision about when to upregulate the expression of QS-controlled traits. According to this hypothesis, cells sense their environment using various mechanisms and encode this information in the rate of AI production - an assumption supported by observations from multiple QS systems (Duan & Surette, 2007; Horswill et al., 2007; Lee et al., 2013; Mellbye & Schuster, 2014; Moreno-Gámez et al., 2017; Slager et al., 2014). Then, by exporting AIs and monitoring their extracellular concentrations, cells can share private estimates of the environmental conditions and gain access to a 'pooled' estimate of the environment. This would allow bacteria to collectively sense their environment and harness the "wisdom of the crowds", a principle observed in animal decision making whereby noise in individual estimates of the environment promotes the use of group consensus (Berdahl et al., 2013; Golub & Jackson, 2010). This functional view of QS is not mutually exclusive with the hypothesis that bacteria benefit from coordinating the expression of certain phenotypic traits through QS. Nonetheless, we show here that this 'collective sensing' functionality of QS systems is sufficient to explain the evolution of QS.

5.2 MODEL

We study the evolution of QS in a population of bacteria facing fluctuating environments where individual estimates of the current or local environmental conditions are noisy. We model bacteria that have the simplest possible internal network of feedback regulation (Fig. 1a). This network consists of a positive feedback loop where a gene product A promotes its own transcription (Supplementary Information). We parametrize this simple network of regulation in a way that there are two possible stable states: an 'OFF' state where A is not expressed and an 'ON' state where there is a high expression level of A. This is a good approximation to many systems where bacteria use QS to regulate the expression of all-or-nothing programs of gene regulation; this includes decisions like sporulating or becoming competent or virulent (Gustafsson, Nilsson, Karlsson, & Arvidson, 2004; Hense & Schuster, 2015; Maamar & Dubnau, 2005; Veening, Hamoen, & Kuipers, 2005). We assume that bacteria can exchange A with the extracellular environment by passive diffusion through the cellular membrane (consistent with how QS works in many Gram-negative bacteria

that do not have dedicated transporters for QS signals (Papenfort & Bassler, 2016)). Hence, A acts both as a product of the gene regulatory network and as a QS signal. In order to study the evolution of cell-to-cell communication, we allow bacteria to evolve a parameter c that determines the permeability of the membrane to A and thus determines the degree of coupling between the cells. In nature, membrane permeability depends on the properties of the AIs and the cell membrane and can change for instance by variations in the length or biochemical structure of the AIs as well as by the evolution of mechanisms to actively secrete AIs extracellularly (e.g. carrier proteins) (Kamaraju et al., 2011; Ng & Bassler, 2009; Pearson, Delden, & Iglewski, 1999).

Bacteria fully occupy a two-dimensional 50x50 grid over which A diffuses at a rate D. Bacteria evolve through a series of environmental cycles that fluctuate randomly between two alternative states, E_{OFF} and E_{ON}, with equal probability (Fig. 1b). In each environmental state there is an optimal level of expression of A for all the individuals in the grid: while E_{OFF} favors bacteria that do not express A, E_{ON} favors bacteria with high levels of expression of A. For instance, E_{ON} could correspond to an environment where bacteria benefit from producing A because a stressor appears and A activates a program of expression to cope with stress (e.g. competence). Activating such a program would not be useful in the absence of the stressor (E_{OFF}) and thus bacteria would benefit from switching off the production of A in this context. At the end of each environmental cycle the fitness of every individual is determined by how well its expression level of A matched the optimal expression level for the current state of the environment, which is denoted as A_{ON} or A_{OFF}. This is, by the absolute difference through the environmental cycle between the value of A and either A_{ON} or A_{OFF} depending on the state of the environment. Then, the grid is fully repopulated by the daughter cells of the fittest individuals at the end of the previous cycle to start a new environmental cycle. The number of offspring produced by every individual is determined based on its fitness relative to the other members of the population. The fitness function has a sigmoidal shape such that individuals are penalized for making a wrong estimate of whether the environment is in the 'ON' or 'OFF' state but not for small numerical deviations from the values of A_{ON} and A_{OFF}, which are set to the two stable states of A when $c = 0$ (Supplementary Information). Upon cell birth, c mutates with probability μ, resulting in c increasing or decreasing by a fixed step size δ with equal probability (subject to the constraint that $c \geqslant 0$).

A key assumption of our model is that bacteria can differ in their individual estimates of the environment despite encountering the same environmental regime and having the same internal network of gene regulation. Such phenotypic heterogeneity has been documented in multiple QS systems (e.g., bioluminescence in *Vibrio*, competence in *Bacillus* and virulence in *Listeria*) where isogenic populations of bacteria that are actively quorum-sensing contain subpopulations of cells in an 'OFF' state (Garmyn et al., 2011; Grote, Krysciak, & Streit, 2015). The origin of these phenotypic differences has been partially attributed to stochastic events at the level of expression of quorum-sensing-related molecules, in particular of AIs, response regulators, and proteins involved in the cascades of QS regulation (Anetzberger, Pirch, & Jung, 2009;

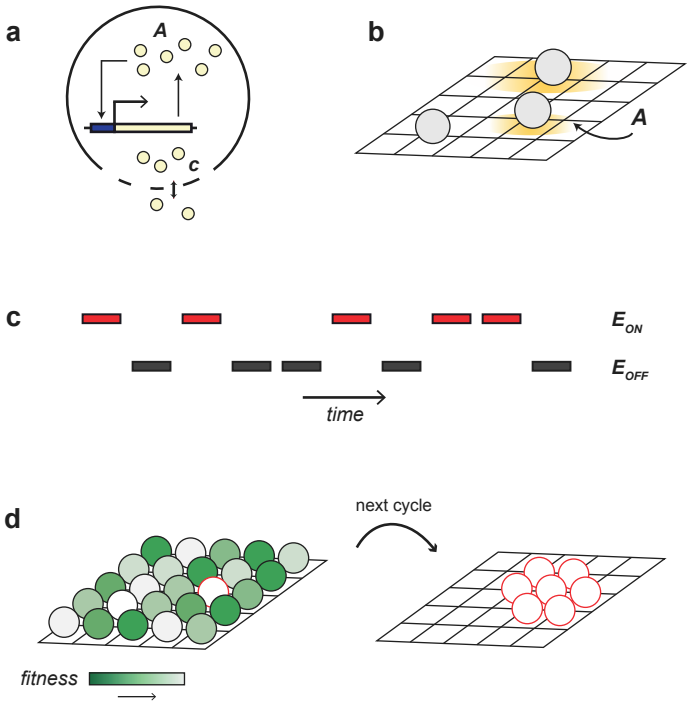

Figure 1: Model structure. a) Internally, a bacterial cell has the simplest network of positive feedback regulation, where A promotes its own transcription. In addition to this gene regulatory network, bacteria exchange A with the extracellular environment by passive diffusion through the membrane at a rate proportional to the evolvable parameter c. b) Bacteria inhabit a two-dimensional grid. For illustration only three cells are shown although the grid is always fully occupied. Every timestep, the intracellular concentration of A is updated for every cell, and the extracellular concentration of A is updated according to a two-dimensional diffusion process with diffusion constant D over the 2-D grid. The yellow halo shows how A leaks from a cell and diffuses over the 2-D grid, and the three cells illustrate different scenarios: (*bottom*) a cell with $c = 0$ that does no exchange A with the extracellular environment; (*center*) a cell that either has a low value of c or lives in an environment where diffusivity D is low; (*top*) a cell with a high value of c that lives in an environment with high diffusivity. c) Bacteria encounter an environment that fluctuates between two states, E_{ON} and E_{OFF}, in an unpredictable manner. While in E_{ON} bacteria maximize their fitness by having a high level of expression of A, in E_{OFF} fitness is maximal if A is not produced. The fitness of every cell is calculated at the end of every environmental cycle as the difference between its level of expression of A through the cycle and the optimal level of expression for the current environmental state. d) Fitness values are then used to determine the number of descendants of every cell (and therefore an environmental cycle is longer than the cell cycle) and in turn to update the grid for the start of the next environmental cycle. The full grid is repopulated in a way that individuals with high fitness leave more descendants and those descendants are placed nearby the original location of their parent. To illustrate this idea the offspring of a cell with high fitness (red circle) is shown from one cycle to the next. Fitness increases from green to white. See main text and SI for model details.

Grote et al., 2015; Pérez & Hagen, 2010; Plener et al., 2015). In our model, we capture this cell-to-cell variation by assuming that at the start of an environmental cycle each bacterium makes an individual estimate of the state of the environment that gets reflected in its internal A concentration. In the model we implement this by letting bacteria sample at the start of each environmental cycle their internal A concentration from a (truncated) normal distribution whose mean is the optimal level of A expression in the current environment (either A_{ON} and A_{OFF}). Sampling from a normal distribution reflects the assumption that bacterial estimates of the current environment are noisy, due to some combination of environmental unpredictability and intrinsic estimation errors. Such noise corresponds to the variance of the distribution.

5.3 RESULTS

Starting from a population where cells make decisions without sharing information ($c = 0$ initially), we find that c increases over time and thus communication readily evolves in the population (Fig. 2). Interestingly, although c increases slowly at the beginning, there are successive sweeps that lead to a rapid transition towards high values of c. This occurs because (i) communication becomes beneficial only after a minimum number of neighboring cells are exchanging information and (ii) these benefits increase with the size of the population that is communicating (Fig. 2 and Fig. S1). Thus, this threshold-like pattern indicates that there is frequency-dependent selection on communication since evolving higher values of c becomes more profitable as more cells in the population have high c.

Exchanging A with the extracellular environment provides cells with information on the initial extracellular concentration of A, which could potentially be beneficial if this concentration is informative of the current environmental state. To avoid that such benefits, which do not result from cell-cell communication, bias the outcome towards the evolution of high c, we implemented initial conditions for the extracellular concentration of A that were uninformative. In particular, we assumed that the initial concentration of A was sampled independently for each grid cell from a uniform distribution with mean $(E_{OFF} + E_{ON}) / 2$. We then simulated the same scenario as in Fig. 2 in the limit of no diffusion of A in the two-dimensional grid ($D = 0$). In this scenario, c remains near zero, showing that in the absence of information exchange between individuals, evolving higher values of c does not provide a benefit and it is actually costly (Fig. S2). This cost arises because in the absence of cell-cell communication the extracellular concentration of A is often deceiving and worsens the estimate that cells made at the start of the environmental cycle. As a result, cells with high c do (on average) worse than cells that rely exclusively on their initial estimate of the environmental state and do not exchange A with the extracellular environment (Supplementary Information).

A consequence of the negative fitness effect of increasing c in the absence of other communicators is that there is a dependency of the evolution of communication

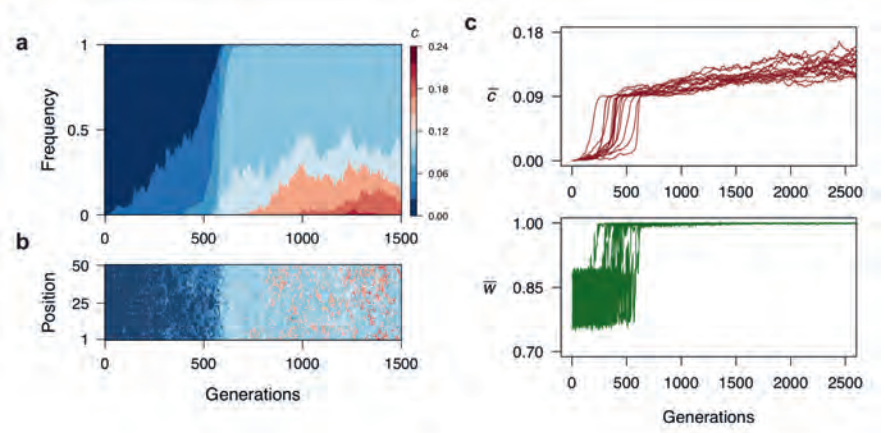

Figure 2: Evolution of QS resulting from its collective sensing functionality. a) Evolution of the communication parameter c across time in a single evolutionary simulation. b) Genetic composition of the bacteria located in a single row of the two-dimensional grid tracked through evolutionary time. Although few cells with increased values of c emerge in the first 100 generations, they are mostly surrounded by cells with $c = 0$ and do not profit from cell-cell communication. At ~500 generations, the amount of cells with $c \neq 0$ increases and there are successive sweeps of higher values of c. c) Mean c (*top*) and mean population fitness (*bottom*) across 2500 generations in 50 replicate evolutionary simulations showing that the rapid spread of communication through the population is associated with a threshold-like increase in the benefit of collective sensing arising once there is a minimum degree of communication in the population. When the mean c exceeds 0.09, most cells are communicating, so fitness increases marginally by evolving even higher values of c. Parameters: $E_{OFF} = 20$, $E_{ON} = 100$, $\mu = 0.001$, $\partial = 0.03$ and $s = 0.8$.

on the size ∂ of the mutational steps. In particular, we find that the evolution of collective sensing is facilitated by intermediate mutational step sizes (Fig. S3): Very small mutational steps slow down the evolution of communication, because it takes very long to reach a minimum cluster of cells exchanging information, whereas too large mutational steps impose a high fitness cost to the first communicators also slowing down the evolution of collective sensing. This cost results from the fact that if a cell is mostly surrounded by non-communicators (as it is the case of the first communicators), the extracellular concentration of A will not be informative and in fact will often be deceiving (given our choice of sampling the initial concentration of A from a distribution with mean (E_{OFF} + E_{ON}) / 2). In this scenario, communication will be costly for the first communicators and this cost will increase the more coupled a cell is to its extracellular environment (i.e., the higher the value of c).

We find that a series of conditions favor the evolution of collective sensing. The first two are related to model assumptions justified previously. First, collective sens-

ing is beneficial only if on average cells make an individual estimate that is close to the current state of the environment (Fig. S4a). Thus, our model is consistent with a general principle of group-making decision theory known as the Condorcet Jury Theorem. This theorem establishes that for a group of individuals using a majority-rule for decision making, the chance of making the right choice increases with the number of voters only if individuals make the correct choice more often than the incorrect one (Boland, 1989). Second, provided that on average individual estimates of the environment are correct, increased noise in the individual estimates of the environment facilitates the evolution of collective sensing (Fig. S4b). In fact, in the extreme scenario where cells could determine the exact state of the environment on their own, there would be no benefit of cell-to-cell communication as a way to improve individual estimates of the environmental conditions. Interestingly, the shape of the noise distribution affects the benefit that bacteria derive from communication. In particular, skewed noise distributions where most of the cells make a good estimate of the current environmental state but a few cells make estimates that are very inaccurate can delay the evolution of cell-to-cell communication (Fig. S4c).

In addition to the previous conditions, we find that two features of bacterial interactions facilitate the evolution of collective sensing. First, in the absence of motility, the offspring of a bacterial cell is often located nearby in space. We study the effect of this feature of bacterial division on the evolution of QS by comparing our findings in which daughter cells occupy a similar or even the same location as their mother cell to simulations where offspring are placed randomly on the two-dimensional grid. Random placement slows the evolution of collective sensing (Fig. 3a,c), in line with considerable literature on the importance of spatial structure in the evolution of collective behavior (Nadell, Foster, & Xavier, 2010; Pepper, 2000; Wakano, Nowak, & Hauert, 2009). This occurs because the benefits of cell-to-cell communication are accrued locally through successive environmental generations. Dispersal frustrates this evolution, both in source habitats of the mother cell and in the target areas to which daughter cells disperse. Second, we find that similar to cell movement, environmental diffusivity also influences the evolution of collective sensing. However, unlike the monotonic negative effect of cell dispersal, extreme high or low diffusion hinders the evolution of cell-to-cell communication. Whereas the absence of diffusion impedes the exchange of information between cells (Fig. S2), highly diffusive environments tend to couple communicating bacterial cells with many non-communicators, diminishing the benefit of local assortment and slowing the evolution of communication (Fig. 3b,c). Thus, the evolution of collective sensing is favored when bacterial cells interact locally.

The patterns identified so far occur in spatially homogeneous environments where the only spatial inhomogeneities that occur are in the form of differences in signaling among cells. How might our results be influenced by realistic environmental gradients, similar to those generated by abiotic or biotic processes? To answer this question we studied the role of spatial heterogeneity in the evolution of communication by modeling different degrees of intermixing of the environmental states E_{OFF} and E_{ON}. During each simulation, contrasting environmental states existed on fixed

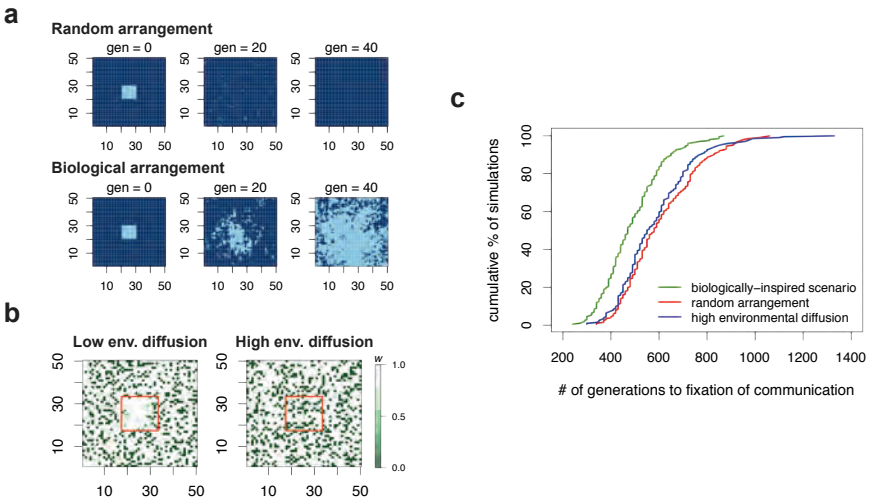

Figure 3: QS, local interactions and the role of environmental diffusion. a) Genetic composition of two populations (shown in the two dimensional 50x50 grid) that start with a subpopulation of communicators (light blue, c=0.09) surrounded by non-communicators (dark blue, c=0) across 40 generations of selection. In the top population, the offspring of a cell is placed randomly in space, whereas in the bottom population, offspring occupy a position close to their mother cell which captures a biological feature of bacterial reproduction. Random placement of offspring leads to the extinction of communicating cells because bacteria with high c only benefit from collective sensing if there are other communicators nearby. b) Individual fitness values in two populations of non-communicators (c = 0) that contain a subpopulation of communicators (c = 0.09, shown by the red square). When environmental diffusion is low (D = 1), the subpopulation of communicators benefits from collective sensing, whereas at high environmental diffusion (D = 10), communicating cells are coupled with non-communicators and the benefit of collective sensing is lost. The fitness values are calculated after one generation where the population encounters an E_{ON} environment. A similar pattern is observed in an E_{OFF} environment. c) Cumulative distribution of the time to fixation of cell-cell communication in three scenarios: (green) biologically-inspired scenario presented in Fig. 2 where the offspring of a cell remains nearby and bacteria interact in a short local range; (red) same scenario as Fig. 2 except that the offspring of a cell is randomly placed on space after reproduction; (blue) same scenario as Fig. 2 except that the rate D of diffusion in the extracellular environment is high (D = 10) so bacteria have a long interaction range. 200 simulations are shown per condition and we define communication as fixed in the population when the mean c surpasses 0.09. The evolution of collective sensing is delayed in the last two scenarios relative to the scenario simulated in Fig. 2. For all panels, unless indicated otherwise, parameters are the same as in Fig. 2.

spatial domains that were created independently for each replicate by a stochastic spatial pattern generator. Each generation, environmental state E_{OFF} existed on one of the two domains with equal probability, and state E_{ON} existed on the other. These spatial domains were generated using an algorithm where pixels of a grid preferably transition to the state occupied by the majority of their neighbors. By running

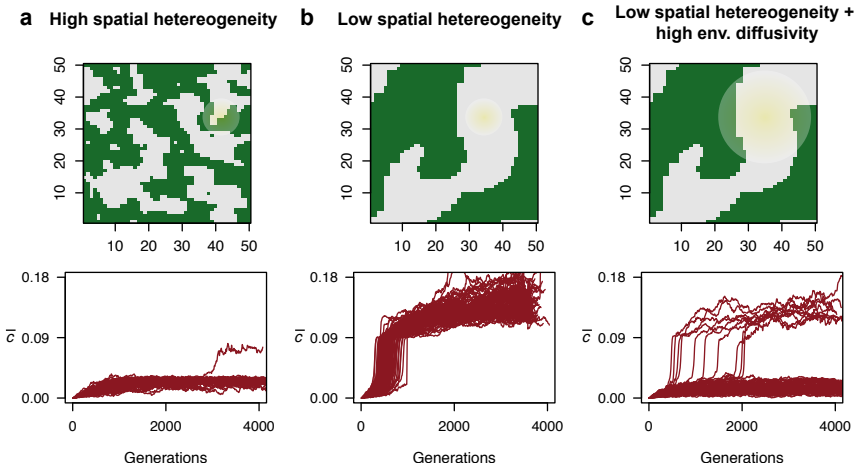

Figure 4: Evolution of QS in spatially heterogeneous environments. a) *(Top)* Example of the spatial domains (green vs. grey reflecting E_{OFF} vs. E_{ON} or *vice versa*) featuring contrasting environmental states in a single evolutionary simulation with high spatial heterogeneity. The yellow halo represents the neighborhood of interaction of a focal cell; the size of this neighbourhood depends on the rate of environmental diffusivity D. (*bottom*) Mean c across 4000 generations in 100 replicate evolutionary simulations with high spatial environmental heterogeneity. Cell-to-cell communication evolves only in a very small fraction of the simulations, because communicating cells receive conflicting information from individuals experiencing a different environmental state. Panel (b) (*top and bottom*) show similar information for a scenario with low environmental heterogeneity. Cell-to-cell communication evolves in all simulations. Panel (c) (*top*) reflects a scenario with low spatial heterogeneity and a high rate of environmental diffusivity, as illustrated by the large size of the yellow halo. Irrespective of the coarse spatial patterning, high diffusion increases the coupling between cells that experience different environmental states, undermining the information value of the external AI signal. (*bottom*) As a result, cell-to-cell communication evolves in only a small fraction of the simulations. Moreover, when it evolves, it takes much longer than in a spatially homogeneous environment.

this algorithm for different numbers of time-steps starting from an initial random configuration of the grid, we were able to generate either fine or coarse-grained domains which we used as a basis for simulating environments with high and low heterogeneity, respectively (Fig. 4; Supplementary Information).

Fine-grained environmental structure generates highly variable information on local scales. As a result, the evolution of collective sensing is strongly hindered in this scenario because cells are exposed to conflicting information from neighbors experiencing different environmental regimes (Fig. 4a). When spatial heterogeneity is low and there is coarse-grained environmental structure this effect also occurs at

domain boundaries. However, cell-cell communication is still beneficial for cells in the center of the spatial domains since they interact with other individuals experiencing the same environmental conditions. Therefore, in contrast to highly structured environments, collective sensing evolves when spatial heterogeneity is low (Fig. 4b). Importantly, these findings are contingent on the size of the interaction neighborhood of an individual, which is set by the rate of environmental diffusivity D. We illustrate this idea by showing that in the same regime with low levels of spatial heterogeneity, high environmental diffusivity can prevent the evolution of communication by increasing the interaction neighborhood of a cell. This makes it more likely that a focal cell is communicating with others that experience a different environmental state, eroding the information contained in the external concentration of A. Thus, when environments vary spatially, the evolution of collective sensing is also favored if bacteria interact at a local scale.

5.4 DISCUSSION

Overall, our model shows that QS can evolve merely as a result of its collective sensing functionality, without the need of a benefit resulting from the coordinated expression of a QS-regulated trait at high population densities. Importantly, this alternative interpretation of QS does not exclude that such benefit exists but rather complements the classical view of QS. In fact, one of the main questions regarding the evolution and widespread presence of QS systems in bacteria is how QS can be stable in the presence of 'cheaters' that do not engage in the collective action carried by the population. One possible explanation is that QS does not only control public functions but also private functions (i.e. functions that only have a direct fitness effect on the cell performing them). The latter are common in many QS systems and a collective sensing interpretation of QS could help explain why they are controlled by QS and in turn how QS remains protected from 'cheaters'.

We note that a potential social conflict can also arise in a collective sensing system if sharing information is costly and independent from receiving information from other cells. This does not apply to our model, where sharing and receiving information are intimately coupled because of our choice of modeling extracellular exchange of A as a passive diffusion process in resemblance of the QS systems of Gram-negative bacteria (Papenfort & Bassler, 2016). More complicated QS architectures, especially the ones of Gram-positive bacteria, where AIs are actively secreted and sensed using different dedicated transporters (Miller & Bassler, 2001; Waters & Bassler, 2005), can have more potential for the emergence of defectors that reap the benefits of communication without contributing to the public signal in situations where communication is costly. This can explain why QS typically involves the production of signalling molecules that are 'cheap' to synthesize and justifies the choice of ignoring such costs in our model.

Importantly, our model shows that the evolution of QS as a collective sensing mechanism could have been particularly favored in bacteria because of two features

of bacterial interaction networks. First, when bacteria divide, their offspring generally end up being located close by in space. This feature can not only protect QS from invasion by cheaters via a kin selection mechanism (Nadell & Bassler, 2011; Schluter, Schoech, Foster, & Mitri, 2016) but as shown here, it can facilitate the emergence of a minimum cluster of communicators arranged nearby in space which is necessary for collective sensing to be profitable. Second, recent evidence suggests that many bacterial interactions occur over short spatial ranges (DalCo, van Vliet, Kiviet, Schlegel, & Ackermann, 2020; Esser, Leveau, Meyer, & Wiegand, 2015).Our model shows that this characteristic of bacterial networks could have favored the evolution of collective sensing because emergent communicators (i) are often coupled to their communicating offspring (Fig. 3) and (ii) avoid long-range interactions with cells located in different microenvironments that could share deceiving information (Fig. 4).

Collective sensing has been proposed as a mechanism for decision-making emerging from social interactions among individuals with rudimentary behavior rules in other biological systems (Berdahl et al., 2013; Cvikel et al., 2015). Our work shows that the same collective functionality can arise in populations of bacteria with simple networks of gene regulation and could have driven the evolution of one of the most widely used communication systems in bacteria: *quorum sensing*.

5.5 ACKNOWLEDGMENTS

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5.6 SUPPLEMENTARY INFORMATION

5.6.1 Supplementary Figures

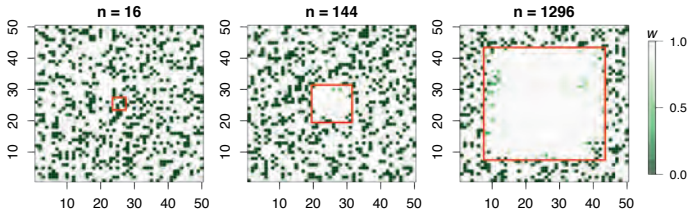

Figure S1: A cluster of cells communicating is necessary for collective sensing to be profitable. Individual fitness values in three populations of non-communicators that contain a subpopulation of cells communicating of different size. In each panel, the size of this subpopulation is indicated by n and its location in the two-dimensional grid is shown with a red square. Cells profit from collective sensing once there is a minimum number of communicators. For non-communicators, $c = 0$ and for communicators, $c = 0.09$. The fitness values are calculated after one generation where the population encounters an E_{ON} environment. A similar pattern is observed in an E_{OFF} environment. The rest of parameters are the same as in Fig. 2.

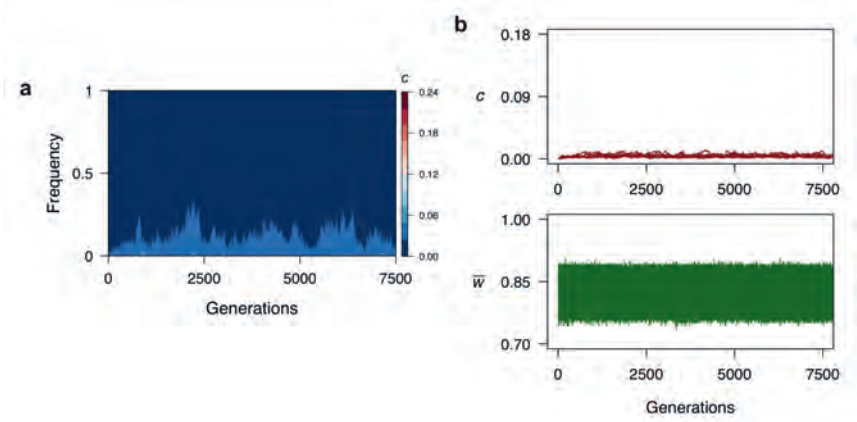

Figure S2: **Communication does not evolve in the absence of extracellular diffusion.** a) Evolution of the communication parameter c across time in a single evolutionary simulation where $D = 0$. b) Mean extracellular diffusion c (top) and mean population fitness (bottom) across 7500 generations in 50 replicate evolutionary simulations. Both panels show that c does not evolve to high values in the absence of environmental diffusion. This shows that cells only benefit from exchanging A with the extracellular environment because they can communicate with other cells and not because they can gather more information on the current environment from the initial extracellular concentration of A. All parameters are the same that in Fig. 2 except from D.

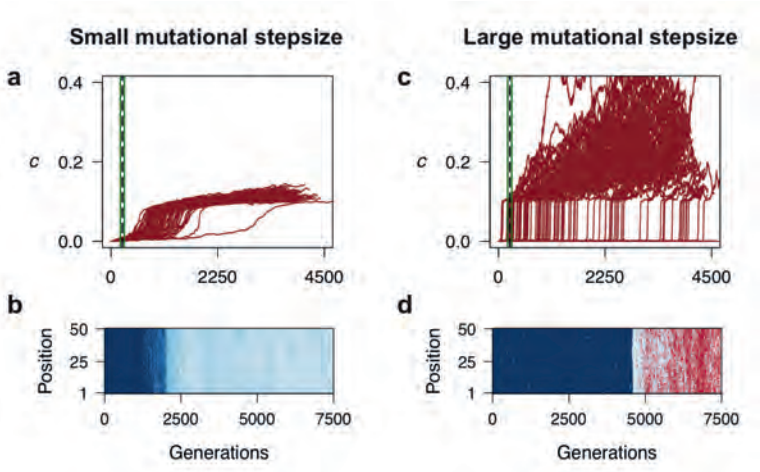

Figure S3: **Intermediate mutational stepsizes favor the evolution of collective sensing.** a) Mean c in 100 replicate evolutionary simulations with the same parameters as in Fig. 2 but a smaller mutational stepsize ($\delta = 0.01$). The mean time for evolution of communication in Fig. 2 ($\delta = 0.03$) is shown by the dotted line with the first and third quartiles shown by green lines. b) Genetic composition of a single row of the two dimensional grid through evolutionary time in one simulation with small mutational stepsize. This illustrates that with a small mutational step it takes very long for a minimum number of communicators with high enough c to emerge relative to an intermediate mutational step (like the one in Fig. 2), which in turn slows down the emergence of collective sensing. c) Mean c in 100 replicate evolutionary simulations with the same parameters as in Fig. 2 but a larger mutational stepsize ($\delta = 0.1$). The solid and dotted lines indicate the same as in panel a). d) Genetic composition of a single row of the two dimensional grid through evolutionary time in one simulation with large mutational step. This illustrates that with a large mutational step communicators arise but go extinct often relative to an intermediate mutational step (like the one in Fig. 2) because in the absence of other communicators high values of c are detrimental since they turn the dynamical system monostable and sensitive to the extracellular concentration of A which is not informative.

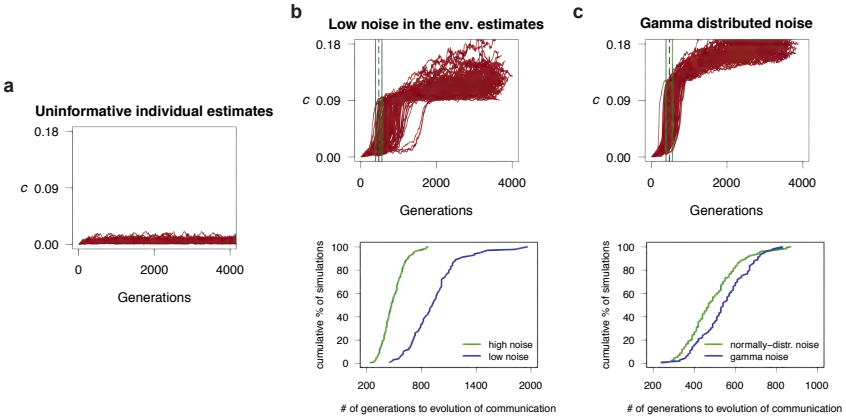

Figure S4: The evolution of collective sensing depends on the amount and structure of environmental noise. a) When bacteria are unable to individually estimate the state of the environment, collective sensing is not profitable and does not evolve. We model this scenario by assuming that bacteria sample their initial intracellular concentration of A from the same distribution regardless of the state of the environment. b) (*top*) Mean c across 3500 generations in 200 replicate evolutionary simulations with the same parameters as in Fig. 2 but with lower noise in the individual estimates of the environmental conditions ($\sigma_{OFF} = 10$ and $\sigma_{ON} = 30$). The mean time for evolution of communication with higher noise in the individual estimates of the environment is shown by the dotted line (Fig. 2, $\sigma_{OFF} = 30$ and $\sigma_{ON} = 80$), with the first and third quartiles indicated by green lines. (*bottom*) Cumulative distributions of the time to fixation of cell-cell communication in the two scenarios compared in the figure above. We define communication as fixed in the population when the mean c surpasses 0.09. Since the benefit of collective sensing comes from the error that cells make when estimating environmental conditions, lower noise in such estimates makes communication less profitable and collective sensing takes longer to evolve. c) (*top*) Mean c across 3500 generations in 200 replicate evolutionary simulations with the same parameters as in Fig. 2 when noise in the individual estimates of the environment has the same mean and standard deviation as in Fig. 2 but is gamma-distributed (as opposed to normally-distributed like in Fig. 2). Vertical lines indicate the same as in (b). (*bottom*) Cumulative distributions of the time to fixation of cell-cell communication in the two scenarios compared in the figure above. As in (b), we define communication as fixed in the population when the mean c surpasses 0.09. When noise in the individual estimates of the environment is gamma-distributed some cells can make very inaccurate estimates of the environmental state. When there is communication, these cells can deceive other cells hindering the evolution of collective sensing.

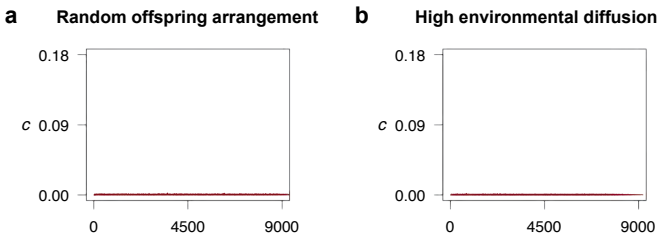

Figure S5: **Random offspring arrangement and high environmental diffusion hinder the evolution of collective sensing especially when mutational stepsizes are large.** Evolution of c across time in a single evolutionary simulation with same parameters as in Fig. S3b ($\delta = 0.1$) but a) when the offspring of a cell is randomly placed in the new grid and b) when there is high diffusion in the extracellular space (D=10). Communication did not evolve during the first 7000 generations in 100 replicate evolutionary simulations of each scenario which shows that both conditions are particularly detrimental for the evolution of communication when the mutational stepsize is large. The negative effect of both of these scenarios on the evolution of communication is amplified by the mutational stepsize because when δ is high, a single mutational step towards high c is highly detrimental if cells are not coupled with other communicating cells (which is not the case when the mutational stepsize is small).

5.6.2 Model description

We study the evolution of cell-to-cell communication in a bacterial population encountering varying environments. The phenotype of a cell is determined by a simple network of positive regulation where a protein A promotes its own transcription (Fig. 1). We model this positive feedback by assuming that the transcriptional regulation of A follows Hill kinetics. Bacterial cells inhabit a two-dimensional grid of size $N \times N$ where they can communicate with other cells by exchanging A with the extracellular space. A is exchanged by passive diffusion with a diffusion constant c. Based on these assumptions the system of equations describing the intracellular concentration of A and extracellular concentration, A_E, is,

$$\frac{dA}{dt} = \sigma \frac{k_0 K_d + k A^n}{K_d + A^n} + c\,(A_E - A) - d\,A \tag{1}$$

$$\frac{dA_E}{dt} = c\,(A - A_E) + D\,\nabla^2 A_E \tag{2}$$

where σ is the number of proteins produced per transcript of mRNA, k_0 is the basal transcription rate when the promoter is not bound to any molecule of A, K_d is the dissociation constant, k is the maximal transcription rate, n is the degree of cooperative binding, d is the rate of degradation of A and D is the rate of environmental diffusion. Since most QS systems exhibit bistability we choose the parameter values in a way that there are two stable states at a high and low concentration of A when $c = 0$ (Fig. S6). The parameter values we chose are $\sigma = 5.14$, $n = 6.75$, $K_d = 1.1 \times 10^{12}$, $d = 0.1$, $k_0 = 0.39$ and $k = 2.0$. We use this set of parameters in all the simulations. Every generation we solve the previous system of equations for a fixed number of time steps T and calculate fitness at the end to determine which individuals will leave offspring in the next generation. We assume periodic boundary conditions for the extracellular diffusion of A.

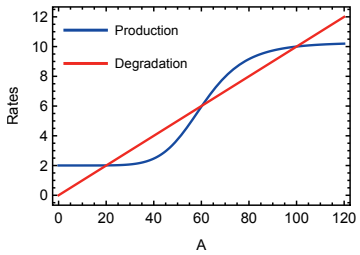

Figure S6: Steady-states of the model with $c = 0$. In the absence of diffusion of A to the extracellular space, we chose a set of parameter values that renders the system described by Eq. (1) bistable. The rate of production of A is given by $\sigma \frac{k_0 K_d + k A^n}{K_d + A^n}$ and the rate of degradation is dA.

5.6.2.1 *Fitness calculation and reproduction*

Every generation a bacterial population faces one of two possible environments with equal probability. Each environment has an optimal expression level of A, denoted by A_{OFF} or A_{ON}. A_{OFF} and A_{ON} are set at the stable equilibria of the bistable system when $c = 0$ (Fig. S6). At the start of a generation all cells sample their initial intracellular value of A from a truncated normal distribution with mean either A_{OFF} or A_{ON} depending on the environment and standard deviation σ_{OFF} or σ_{ON} .The fitness of a cell is determined by how well its intracellular A concentration matches the state of the environment throughout the duration of a generation. The fitness function is,

$$w(\Delta_E) = \frac{1}{1 + e^{s\,(\Delta_E - x)}}$$

where s determines the strength of selection, $\Delta_E = \frac{1}{T}\sum_{t=1}^{T} A_t - A_{ON}$ (*i.e.* the average difference over the T time steps between A and the optimal level of A expression in the current environmental state, in this example E_{ON}) and x is the the midpoint of the sigmoid curve. For all simulations we set $s = 0.8$ and $x = 25$. For this choice of parameters w has a sigmoidal shape that strongly penalizes cells that are in the incorrect phenotypic state but not cells that slightly deviate from the optimal expression levels (Fig. S7).

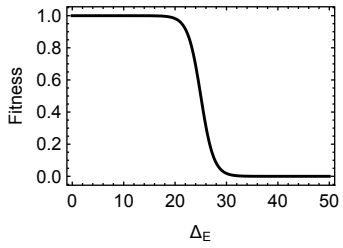

Figure S7: Fitness function w**.** Function applied at the end of one generation to determine the fitness of a cell depending on the difference Δ_E between its average value of A and the optimal expression level for the current environmental state.

At the end of a generation the fitness of every cell is calculated and fitness values are normalized by the total fitness of the population. Then, $N \times N$ cells are randomly sampled using the normalized fitness values to populate the new grid. The algorithm for creating and placing the offspring of a cell in the new grid is the following,

1. Draw a random number to determine if c mutates. If c mutates, draw an additional random number to determine whether the new value of c is $c + \delta$ or $c - \delta$. If $c - \delta < 0$, c does not mutate.

2. Calculate distance of the mother cell to all other cells in the grid. We use a euclidean distance metric.

3. Place the new cell in the closest grid cell to the mother cell that is still empty.

This algorithm is applied to the $N \times N$ vector containing the coordinates of the cells that will reproduce and it ensures that the offspring of a cell remains close to the location of its mother cell. In simulations where the offspring of a cell is randomly placed on the two dimensional grid, the grid is filled by rows in the order that cells appear in the $N \times N$ vector.

5.6.2.2 *Spatial heterogeneity*

We model variations in the environmental conditions on space by using an Ising model (Onsager, 1944) to establish the initial configuration of the environment. Using this model we can vary the scale of spatial heterogeneity from a random configuration to a homogeneous grid. In two dimensions, this model consists of a grid where cells can be in two possible states (-1 or +1). The total energy of the system is determined by whether neighboring cells are in the same or in different state and is given by,

$$H = -J \sum_{<ij>} s_i s_j$$

where $< ij >$ denotes all the pairs of neighboring cells, s_i is the state of the grid cell i and J determines the sign of the interaction. We assume that $J > 0$ so over time the system converges from a random configuration to a configuration where all the cells have the same state.

Starting from a random configuration where each grid cell is assigned to any of the two states with equal probability, we simulated this model using a Metropolis Monte Carlo algorithm for I number of iterations (Adler, 2010). Briefly, each iteration a grid cell is selected at random and its state is flipped. If the energy of the new configuration is lower than the energy of the old configuration the change in state is accepted and the state of the cell is flipped. If $\Delta E \not< 0$, the state of the cell can still be flipped with probability $e^{-\Delta E / S_T}$, where S_T is a scaling constant. In every iteration, this is repeated $N \times N$ times. Given that the grid configuration will reach equilibrium when all grid cells are in the same state, we can vary the scale of environmental heterogeneity by modifying I.

For each simulation run we first determine the configuration of the $N \times N$ grid by running the previous algorithm for I iterations. We use the resulting grid configuration made of the two states, -1 and +1, to determine the state of the environment in each grid cell every generation. At the start of every generation a random number is drawn to assign the environmental states to the grid states. E_{OFF} and E_{ON} are assigned to grid cells -1 and +1 or vice versa with equal probability every generation. Every bacterial cell samples an initial intracellular A concentration from a distribution whose mean is determined by the environmental state in the grid cell inhabited by the bacterium.