OPINION

Does efficiency sensing unify diffusion and quorum sensing?

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Abstract | Quorum sensing faces evolutionary problems from non-producing or over-producing cheaters. Such problems are circumvented in diffusion sensing, an alternative explanation for quorum sensing. However, both explanations face the problems of signalling in complex environments such as the rhizosphere where, for example, the spatial distribution of cells can be more important for sensing than cell density, which we show by mathematical modelling. We argue that these conflicting concepts can be unified by a new hypothesis, efficiency sensing, and that some of the problems associated with signalling in complex environments, as well as the problem of maintaining honesty in signalling, can be avoided when the signalling cells grow in microcolonies.

In a confined space, bacteria can change their environment in their favour. In an open and mixed environment, by contrast, bacteria must cooperate to carry out these functions, such as the production of a sufficient concentration of exoenzymes to digest complex organic matter[1]. In addition, the cooperation of a group of cells can change the environment on a larger scale[2] and enable concerted actions such as attacking self-defending host tissue[3], defending against predators (protozoa or immune cells[4]) and fruiting-body formation[5,6].

Although direct experimental support for the usefulness of cooperation often lags behind the number of postulated cases, the intriguing idea that some cooperative activities are only effective if a sufficient density of cells is available for coordinated action, and that cell–cell communication could be used for this coordination, has received much attention. Before we explain the conflicting hypotheses concerning the function of this type of signalling (in historical order), we will summarize the common ground of both hypotheses: the molecular mechanisms of signalling and the signal molecules involved.

Bacterial cells produce small, diffusible signalling molecules that are secreted into the environment, from which they can leave by diffusion or advection. The producer cells respond to their own signals, which are therefore called autoinducers[7–9]. Typically, an autoinducer induces the transcription of a set of genes that includes the gene encoding the autoinducer-producing enzyme, which results in a positive feedback loop[10]. The autoinducer can accumulate to sufficiently high concentrations to trigger a response by the cell if the rates of autoinducer production, decay and mass transfer integrated over time reach a threshold concentration at the cell's location.

Although autoinducers are all small, diffusible and metabolically relatively inexpensive molecules, they belong to many different classes of chemicals. *N*-acyl-L-homoserine lactones (AHLs) are the best-studied class of autoinducer, but are only found in Gram-negative bacteria of the phylum Proteobacteria[10,11]. Oligopeptides are the typical autoinducers in Gram-positive bacteria[12,13]. The more recently discovered autoinducer 2 (AI-2) is a mixture of *S*-adenosylmethionine-derived furanones in chemical equilibrium. AI-2 has already been found in many different bacterial taxa, including Gram-positive and Gram-negative organisms, and has therefore been suggested to be a universal signal for communication across species[14–16]. Many more compounds have been identified than can be mentioned here. For the purposes of this article, the chemical nature of the autoinducer is only relevant if there are differences in its specificity or the costs of production, and so we will mainly use the generic term autoinducer.

Quorum and diffusion sensing

Because all the early known responses of bacteria to high autoinducer concentrations, such as interaction with animal or plant hosts, seemed to make sense only when carried out as a concerted action by a group of cells, Fuqua *et al.* coined the term quorum sensing (QS), defining this "minimum behavioral unit as a quorum of bacteria"[17]. It should be noted that the idea of a minimum behavioural unit implies that the purpose of autoinducer sensing is twofold, both taking a census (has the minimum density for effective action been reached?) and coordinating or synchronizing behaviour, so that the quorum of bacteria functions as a unit[18].

However, the 'decision' of the bacteria to alter behaviour when a quorum has been reached is not based on perfect information. The autoinducer concentration that could function as an estimate of cell density is altered by many factors, including diffusion and advection, spatial distribution, degradation and the production of the same autoinducer by third parties, whether intentionally or by chance. As the bacteria cannot estimate any of these processes independently, they cannot correct their estimate of cell density to account for these factors. In fact, they might as well use the autoinducer concentration as an estimate of any of the factors that can affect the concentration of this molecule, such as diffusion limitation. This realization led Redfield to propose in 2002 that quorum sensing is in fact diffusion sensing (DS)[19], in which the function of secreted autoinducers is to determine whether secreted effectors would rapidly diffuse away from the cell, thereby allowing bacteria to detect situations in which the disappearance of effectors

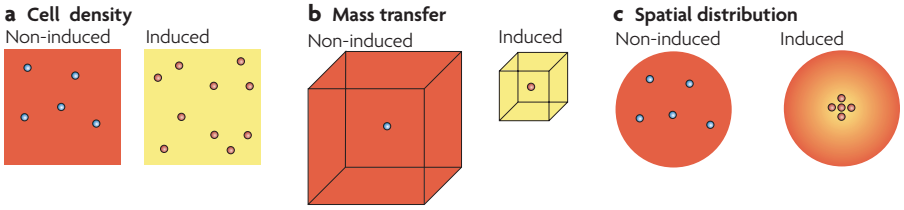


Figure 1 | **What cells sense.** The 'information' that cells obtain from autoinducer sensing is a result of the interaction of autoinducer production with environmental conditions and the density and distribution of the producing cells. **a** | The autoinducer concentration in a well-mixed liquid, or any other habitat without limitations to mass transfer and without clustering of cells, measures the cell density. A sufficiently high cell density leads to upregulation of gene expression. **b** | The concentration of autoinducer produced by a single cell measures the degree to which the mass transfer of this autoinducer is reduced by solids, gas bubbles, or other abiotic or biotic obstacles to mass transfer, and by the composition of the matrix affecting diffusivity and flow velocity. A sufficiently confined space leads to upregulation of gene expression. The cube indicates the volume of the confined space. **c** | The autoinducer concentration in the absence of mass-transfer constraints measures the degree of clustering of a given number of cells. A sufficiently aggregated spatial distribution of cells leads to upregulation of gene expression. A red or yellow background indicates low or high autoinducer concentration, respectively; bacteria in cyan are not induced, and bacteria in red are induced.

owing to diffusion is so low that effector secretion is efficient. The more general term 'mass transfer' can be used instead of diffusion if losses can also occur through advection.

The concepts of QS and DS translate ideas of what factors bacterial cells sense (FIG. 1) directly into hypotheses about the function of sensing, or why bacterial cells sense (TABLE 1). Furthermore, the QS and DS concepts also encompass alternative evolutionary hypotheses. QS postulates that bacteria sense their density to allow them to engage in social behaviour; accordingly, QS assumes that sensing evolved because of the group benefits of social behaviour. DS proposes that sensing is an autonomous activity of single cells to detect mass-transfer limitation; accordingly, DS assumes that sensing evolved because of a direct fitness benefit for the individual. DS, which was proposed after QS, is the simpler hypothesis, as it does not invoke social behaviour and group benefits for the evolution of autoinducer sensing.

How cells interpret autoinducer levels is not merely of academic interest. Understanding when and how bacteria can benefit from the production of autoinducers could translate into knowing when and how to interfere with sensing for our advantage, for example, to foster plant-growth-promoting rhizobacteria[20] in agriculture or to eradicate chronic biofilm infections in patients[21] or unwanted biofilms in industry[22]. After all, many virulence factors of plant and animal pathogens are regulated by autoinducers, as are interactions with plant pathogens in the rhizosphere and phylloplane[23,24].

In this Opinion, after an overview of the problems facing autoinducer-sensing bacteria in natural systems rather than in the laboratory, we will discuss whether the original QS or the alternative DS concept is the most appropriate interpretation of autoinducer signalling. Using a mathematical model, we will show that the spatial distribution of cells can be more important than their density, and that spatial distribution and density are independent measures. As a consequence, we introduce efficiency sensing (ES) as a unifying functional hypothesis for autoinducer signalling that acknowledges the fact that autoinducers can only measure the combination of cell density, limitations to autoinducer mass transfer, and spatial distribution. ES is also a unifying evolutionary hypothesis as it argues that sensing will have been favoured by both individual and group benefits. Finally, we show that the typical mode of growth of attached bacteria — the formation of clonal clusters (microcolonies) — avoids problems of complexity and cheating that autoinducer-sensing bacteria encounter *in situ*.

Problems associated with QS

This section examines a plethora of problems that QS faces *in situ*. We divide these problems into problems of complexity and cheating. The complexity problems are further divided into those arising from spatial heterogeneities in the environment and those arising from biodiversity, because these different classes of problems do not

Table 1 | **Quorum sensing, diffusion sensing and efficiency sensing**

Concept	Hypothesis		
	QS	DS	ES
How cells sense	Individual cells emit small diffusible autoinducer molecules that are sensed by themselves and others, leading to regulation of gene expression. We refer to the mechanism of sensing as autoinducer sensing if we do not want to imply what and why cells sense and who benefits from this.		
What cells sense	Cell density (or, less accurately, the cell number). An ensemble property on the population scale, not only defined at the positions of individual cells.	Mass-transfer properties of the environment surrounding a focal cell. Independent of cell density and spatial distribution.	A combination of cell density, mass-transfer properties and spatial cell distribution as the cell cannot determine density, mass transfer or clustering alone.
Why cells sense	To detect situations in which cell density is sufficient to make a coordinated response of a group of cooperating cells worthwhile.	To detect situations in which mass transfer is sufficiently limited for single cells to respond by producing extracellular diffusible effectors.	To estimate the efficiency of producing extracellular diffusible effectors and to respond only when this is efficient*.
Benefit	Hypothesis suggests QS evolved because of group fitness benefits.	Hypothesis suggests DS evolved because of individual fitness benefits, making DS a simpler hypothesis than QS.	Hypothesis suggests ES evolved because of both individual and group fitness benefits. Both work in the same direction, yielding broader conditions under which ES would be selected for.

* In efficiency sensing, the ecologically relevant information is the combination of all factors that affect autoinducer and effector concentrations in the same way. Cooperative sensing and effector production unavoidably emerge if more than one cell with the same autoinducer system is present. DS, diffusion sensing; ES, efficiency sensing; QS, quorum sensing.

apply to DS and ES in the same way as they apply to QS. For simplicity, we will focus on problems inherent to autoinducer signalling, and ignore the fact that autoinducers can also have functions unrelated to signalling, such as the role of LuxS (the enzyme producing the AI-2 precursor) in methyl metabolism[25–27], and the fact that the products of chemical, and therefore unavoidable, breakdown of AHLs can also have functions independent of signalling[28].

Spatial heterogeneity. Although the concept of QS arose from investigations of clonal bacterial populations growing in well-mixed liquid cultures, in nature most of the sensing takes place in highly diverse communities that are living in temporally fluctuating, spatially heterogeneous environments, such as soil. The rhizosphere can be considered to be a suitable test case for the applicability of the QS concept in natural systems as it exemplifies many of the problems that arise from the temporally changing, complex spatial structure of diffusion spaces and the temporally changing spatial distribution of cells that produce a given autoinducer at a basal or induced rate[29–31] (FIG. 2). Cell clusters of various sizes are common as they arise wherever sufficient nutrients become available for an attached cell to grow and divide. Many bacteria in the rhizosphere grow in depressions, grooves or pockets in soil particles or roots, often in a biofilm matrix that is surrounded by barriers to diffusion, such as solid and gaseous phases, and that can reduce diffusivity itself. Mathematical modelling demonstrates that the autoinducer concentration at the cell's location can depend more strongly on the spatial distribution of the producing cells than on their density (BOX 1).

Clusters of cells are of major importance for signalling. This is supported by experiments in the rhizosphere of wheat, using *Pseudomonas putida* strains engineered to either produce or sense an autoinducer[32]. Model-based analysis of confocal laser scanning microscopy data indicated that the autoinducer concentration is dominated over a wide spatial range by bacterial cell clusters, which, owing to positive feedback, produce autoinducers at the higher rate of induced cells[32]. In oral biofilms under flow conditions, signalling mainly occurs within rather than across clusters[33]. Under typical conditions, in our opinion, it is therefore impossible for bacterial cells to measure their density as such. This is in contrast to the 'quick definition' of QS as the control of gene expression in response to cell density[34], but it does not conflict with the view of QS as coordinating the actions of a group of cells[18].

Biodiversity and interference. Apart from the problems arising from spatially constrained mass transfer and irregular spatial distributions of cells, the high local diversity of species in natural environments facilitates a multitude of interactions that seem to be communication between different bacterial strains and species. Such so-called cross-talk[35–44] can just happen by chance, in which case the partners will be unknown and unpredictable and the signal might not therefore convey useful information, or it can happen because it evolved for this function.

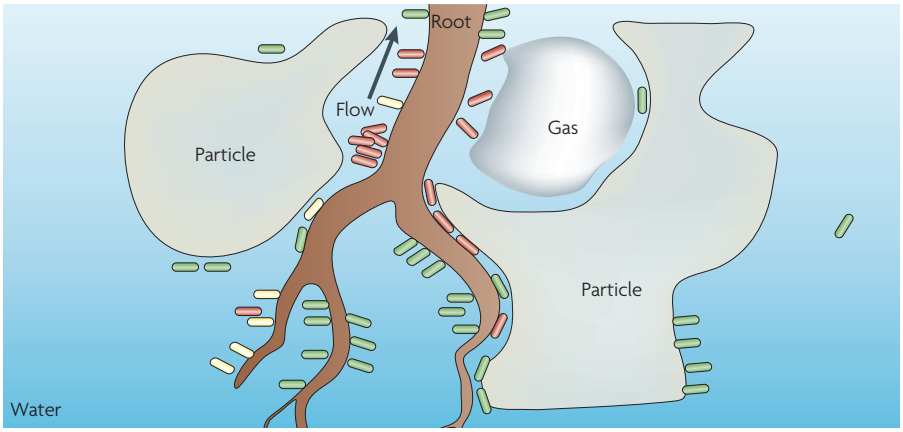


Figure 2 | **The rhizosphere as an example of a complex habitat.** The rhizosphere is characterized by a spatially structured environment that is subject to temporal fluctuations, high biodiversity and a distinct pattern of spatial distribution of individual members of the various bacterial species present. Clear rods represent individuals of a bacterial species producing a certain autoinducer and other, potentially interfering species are indicated by red and green coloured rods. Members of a species are more or less scattered and can occur as single cells or in microcolonies, which are often clusters of cells originating from a single ancestor (clones).

Cross-talk on purpose can be divided into cues, chemical manipulation and signals[45]. A further complication arises from the chance presence of non-producing cells that can function as a barrier to both signalling and cross-talk[46].

Cross-talk can be hard to avoid. In the case of AHLs that are used by Gram-negative bacteria, the chemical diversity of these autoinducers is rather restricted (probably fewer than 100 different structures[10]) in comparison to the bacterial diversity in soil and marine sediments, which is several orders of magnitude higher[47–51] (even considering that only ~10–20% of cultivable soil isolates produce AHLs[37,52]). AHL-based cross-talk will therefore be common in environments with high bacterial diversity. In the case of AI-2, which has been postulated to function as a universal signal[14–16], cross-talk is proposed to be the function for which AI-2 signalling evolved. However, it is difficult to prove that AI-2 is universal as there are many bacterial lineages with few if any cultured representatives, and the evidence for perception, rather than mere production, of AI-2 has been disputed[26]. The usefulness of 'talking' to a more or less random, and therefore unpredictable, set of species with a wide range of metabolic and competitive characteristics is questionable, in our view. Also, it needs to be demonstrated that potentially cross-talking species, such as *Vibrio harveyi* and *Porphyromonas gingivalis*[53], are actually 'within earshot'; methods to analyse such 'calling distances' have recently been developed[54].

In the case of the oligopeptide autoinducers that are used by Gram-positive bacteria, the potential chemical diversity of these compounds, in contrast to that of AHLs, is sufficient to enable species-specific cell–cell communication[12,13]. Therefore, for Gram-positive bacteria, the problems caused by unintended cross-talk will be diminished, but cues and chemical manipulation based on specific chemical autoinducers are nevertheless possible.

Often, so-called cross-talk is actually chemical manipulation or interference with the measurement of the level of autoinducer produced by a given population. For example, blocking autoinducer perception[55–57], autoinducer degradation[3,52,58–63] or autoinducer uptake[64]; producing or mimicking the autoinducer [43,56,65–67]; producing a self-strain-activating and cross-strain-inhibiting autoinducer[68]; or producing a pheromone that is toxic to other species[69]. These types of interference could be expected to prevail

in microbial communities that have a high diversity. However, they are costly, requiring the production of autoinducer antagonists, autoinducer degrading or transporting enzymes or the production of large amounts of autoinducer. It has been shown that interference involving mechanisms that incur a net fitness cost cannot turn a superior resource exploiter into an inferior competitor[70]. If interference involves mechanisms that provide a net benefit, for example by using autoinducers produced by competitors as a substrate for growth, it allows the species that is superior at interference to coexist with, but not dominate, a superior resource exploiter[70]. Therefore, the range of conditions under which autoinducer interference is advantageous might be limited. Further, one way to avoid interference would be to take an independent measurement with a second autoinducer — preferably from a different class of chemical — and compare the results. This idea of coincidence detection[71] would at least allow the cells to detect interference as a result of additional autoinducer sources. Nevertheless, unintended cross-talk and interference are problems that QS will face *in situ*, and one might wonder whether QS works in complex microbial communities.

The evolutionary stability of QS. The chemical manipulation between different species discussed above resurfaces in the context of the evolutionary stability of cooperation because within a population of a certain bacterial species, mutants can arise that manipulate others into increased production of public goods, which include autoinducers and effectors. The evolutionary problem posed by these signal over-producers is that of maintaining honesty in signalling (BOX 2). Signal over-producing mutants and mutants that do not produce public goods can out-compete cooperating individuals, as cooperating individuals pay for the production of public goods that also benefit the non-cooperating mutants, collectively known as cheaters.

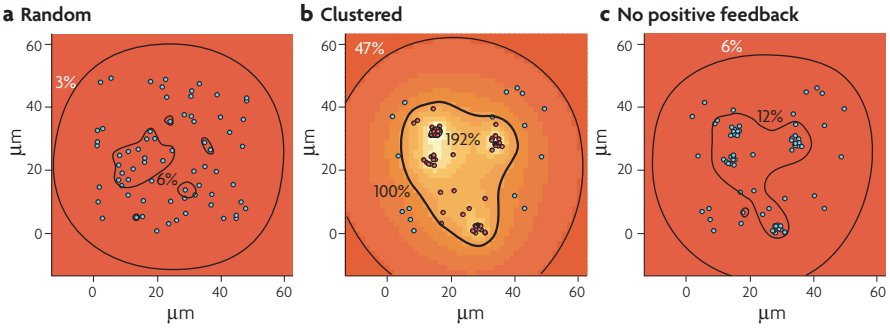
It is a fundamental problem in evolutionary theory to explain how cooperation can evolve in the face of cheaters that have a higher direct fitness because they benefit from public goods without paying the cost of producing them. The key to understanding this is that cooperative effort can be preferentially directed towards relatives (kin selection), which will increase the fitness of the relatives and, indirectly, the fitness of the cooperating individuals because there is a higher than average probability that they will have the same genes. Preferentially directing benefits towards relatives could be achieved by kin discrimination, but this is difficult to achieve in the case of public goods[72]. Directing benefits to relatives is easier if cells are growing in a microcolony, thereby maintaining a clonal spatial structure so that the public goods produced by an individual primarily benefit the direct neighbours, which tend to be relatives. Ironically, the QS concept does not consider this spatial structure, which is key for the evolution of cooperation, including QS. For more in-depth recent reviews, see REFS 45,72–76.

Given the problems of complexity and cheating that are associated with QS *in situ* outlined above, it is surprising to us that QS seems to work at all, yet the existence of autoinducer systems in bacterial isolates from nature, including infected plant and animal tissues, indicates that it does. In the following sections, we will first discuss whether what is commonly regarded as QS can be interpreted in different ways and whether these alternative concepts face the same problems, and then suggest that microcolonies solve the problems of complexity and cheating in autoinducer sensing, regardless of how it is interpreted.

DS versus QS

As a rule, autoinducer-regulated phenotypes are associated with the release of diffusible effectors. For example, bacteria need exoenzymes to degrade macromolecular material as they cannot take up macromolecules

Box 1 | **The importance of spatial clustering of signalling cells**



We developed a generic mathematical model for autoinducer systems with or without positive feedback, which can be applied to *N*-acyl-L-homoserine lactone (AHL) and other autoinducer systems; the parameters used for the examples shown, however, were based on AHLs. The mathematical model was used to calculate the equilibrium AHL concentrations for various spatial patterns, based on the following assumptions. The time unit was chosen in such a way that the diffusion constant of AHL is 1 μm^2 per time unit. Each cell is a point source of AHL molecules, producing AHL molecules with a basal rate of one molecule of autoinducer per time unit if the AHL concentration at the location of the cell is below a certain threshold, and a 100-fold higher induced rate above this threshold. This is on the conservative side of the ratios of induced and basal synthesis rates of the primary autoinducer in *Pseudomonas aeruginosa* cultures (150-fold in serum medium and >370-fold in Luria-Bertani (LB) medium), as estimated by fitting a kinetic model to time-series data[92]. The threshold concentration was assumed to be 50 nM[10], which is equivalent to 30 molecules per cell, assuming that the cell volume is 1 μm^3. Abiotic degradation of AHL molecules has not been included for simplicity (decay is insignificant for AHLs at pH 7.0, at which the half life is about one day[93], nevertheless, for certain autoinducers at certain pH values, the rate of chemical decay can be significant). For the random arrangement, a uniform probability distribution was used (every position equally likely). The qualitative outcome is not affected by changes in the values of the parameters within a reasonable range (data not shown).

We used this model to investigate the effect of the spatial arrangement of autoinducer-producing cells on the local autoinducer concentration reached. Comparing a random (**a**) with a clustered (**b**) arrangement of the same number of cells (70 cells in a volume of $50 \times 50 \times 10$ μm^3 on a solid surface), it is evident that the threshold concentration for induction is only reached in the clusters and in their vicinity. Comparing the same clustered pattern with (**b**) and without (**c**) positive feedback in autoinducer production shows that this typical characteristic of autoinducer production is crucial for reaching sufficient autoinducer concentrations for cells to induce autoinducer production and other genes.

The figure shows bacteria that are not induced (subcritical) in cyan and those that are induced (supercritical) in red. The autoinducer concentration, as a percentage of the threshold concentration, is indicated by contour lines and background colour, for which a linear colour map from red (<16%) to white (>200%) was used. In **b**, the thick contour line separates the subcritical from the supercritical concentrations. The 3D domain is viewed from the top, onto an impermeable surface at the bottom. As the domain is otherwise infinite, the autoinducer can diffuse away.

directly. Such extracellular products will not accumulate to sufficient concentrations to be effective if they disappear by diffusion, advection or enzymatic or chemical degradation. The DS hypothesis suggests that bacteria avoid the costly production and secretion of these substances under conditions in which they would be lost by diffusion or other mass-transfer processes[19]. If the bacterial cell produces and releases small amounts of an autoinducer molecule that it can sense with high sensitivity, it can distinguish situations in which loss from mass transfer is minimal from situations in which it is high and the production of effector molecules would be wasteful. Redfield proposed that the function of autoinducer sensing is not to measure cell density, as postulated by the QS concept, but instead to measure the degree to which secreted effectors would diffuse or flow away[19]. If autoinducers were used by individual cells to measure diffusion or, more generally, the mass-transfer properties of their environment, then autoinducer sensing would be an autonomous rather than a social activity, and selected because it directly benefits the individual rather than because of group benefits (TABLE 1). An example that can be interpreted as diffusion sensing is when one to a few *Staphylococcus aureus* cells producing the Agr autoinducer in the endosome of mammalian cells fill this confined compartment with autoinducer until a crucial concentration is reached[77].

However, there are interesting exceptions to the rule that signalling induces the production of diffusible effector molecules. *P. aeruginosa* downregulates the type III secretion system, which is used for direct injection of virulence factors into host cells, at high cell density[78]. Type III secretion would not be effective in a biofilm where the neighbours are bacteria rather than host cells, so turning off type III secretion at high cell density would make sense if high cell density correlates well with being in a biofilm. Bioluminescence, which led to the discovery of autoinducers and the concept of QS, is another example of a sensing-regulated phenotype that does not involve the release of diffusible effectors as light is produced enzymatically inside the cell. Bioluminescence is only effective at a high cell density because visibility requires many light-emitting bacteria. In these examples, the function of autoinducer sensing is presumably not the detection of mass-transfer-limited regimes.

Box 2 | **The problem of maintaining honesty in signalling**

Signalling systems face the evolutionary problem of honesty as the sender has an incentive to pretend that the population density is higher than it actually is if this manipulates the recipient into producing costly effectors in the absence of a quorum or before the quorum has been reached. The recipient has no means of checking the conveyed information and therefore cannot punish dishonest senders. That is why maintaining honesty requires that any deviation from equilibrium signalling levels should be costly for the sender[94]. For example, higher signal production incurs higher costs[95]. The costs of signal production (and of the signal production machinery) depend on the chemical nature of the autoinducer and can be low[45,96]. However, in addition to this accounting cost, there is the opportunity cost of forgoing the opportunity to use the autoinducer (or rather the resources that went into its synthesis) as a food source (bacteria can use autoinducers as a food source[58]), rather than secreting it into the environment.

The evolutionary stability of the two linked traits, cooperative signal production and cooperative signal response, has been analysed with the help of a simple mathematical model of signalling which assumes that signal and effector production are costly, that the fitness of the group increases with total effector concentration and that an increase in signal concentration elicits an increased rate of effector production[95]. The model predicts the highest signalling intensity at intermediate relatedness because the individuals signal intensely to manipulate their competitors into greater and earlier acts of cooperation. To our knowledge, the predictions of this model have not been tested experimentally. However, the occurrence of autoinducer systems that control the production of public goods in many investigated bacteria[37–39] testifies to the evolutionary stability of these systems under many conditions.

Note that not all signal-deficient or response-deficient mutants are cheaters. Responding to the signal, for example, by producing effectors, might simply be disadvantageous under certain conditions, favouring the evolution of signal-blind mutants because they do not respond anymore, and not because they cheat. For example, *Pseudomonas aeruginosa* signal-blind *lasR* mutants are significantly more resistant to cell lysis and cell death than the wild type under alkaline conditions in stationary phase[97]. Further, in long-term infections of the lungs of cystic fibrosis patients with *P. aeruginosa*, signal-blind *lasR* mutants are common[78,98]. Apparently, these signal-blind strains are fitter as they out-compete the wild type under these conditions. Signal-blind mutants are apparently less fit in changing environments, as ~80% of all environmental and clinical isolates of *P. aeruginosa* are signalling competent[97].

The relationship between the QS and DS hypotheses with regard to what the cells sense (cell density or mass-transfer properties) is straightforward to analyse mathematically. FIGURE 3 illustrates that cell density and mass-transfer sensing correspond to extreme cases based on the same spatial arrangement of cells. In the case of cell density, the threshold for the upregulation of cells can be related to the critical distance between cells in a domain without mass-transfer barriers (for example, planktonic bacteria in a well-mixed liquid). In the case of mass transfer, the same threshold for upregulation can be related to the side length of a box that would be filled by an equivalent equilibrium concentration of autoinducer produced by a single cell. The critical distance for cell density and the box length for mass transfer are the same in the geometrically simple case of cells located at regular, equidistant locations (FIG. 3), but the same argument holds for random cell locations when the critical distances are interpreted as average distances (however, for fixed cell positions, cells associated with clusters formed by chance could be upregulated sooner).

The relationship between QS and DS with regard to the proposed function of sensing, as well as the triggered response to sensing, is also straightforward to analyse. The concept of QS is based on the view that bacteria cooperate and coordinate their activities. In fact, these simple organisms show many examples of social behaviour[2,24,73,74,79–84], but this alone does not prove that autoinducer sensing is also social behaviour. Nevertheless, let us suppose that autoinducer sensing is social behaviour, then as a consequence, social strife cannot be avoided. Therefore, experimental demonstrations of the differential fitness of cheating mutants compared with the cooperating wild-type would support the QS hypothesis that autoinducer sensing is social behaviour[19,76]. By contrast, the concept of DS is based on the view that individual bacteria sense the 'diffusion space' around them by releasing diffusible test molecules. This view is simpler, as DS is an autonomous activity of single cells, not social behaviour. Occam's razor would favour DS as the simpler concept, which does not have to invoke fitness benefits at the level of the group to explain the evolution of the sensing mechanism[19,76].

Earlier, we divided the practical problems of autoinducer sensing into those arising from biodiversity and those arising from spatial heterogeneities in the environment. The problems arising from the local presence

of other populations, such as unintended cross-talk and interference from competitors, distort the measurement of both cell density (the supposed function of QS) and mass-transfer limitations (the supposed function of DS). The problems arising from the patchiness of habitats, however, only distort or invalidate the measurement of cell density (QS), whereas the often complex spatial structure of the environment leads to variations in mass-transfer properties from patch to patch, which is exactly what DS is supposed to measure: the autoinducer is produced to test whether advective flow would wash away, or high diffusivity would dilute out, any products that are released from the cell. The spatial complexity of the habitat is therefore not a problem for DS, but its purpose.

Spatial distribution versus QS and DS

The QS concept posits that the function of the autoinducer-sensing mechanism is to measure cell density. It is clear from the example in BOX 1 that the spatial distribution of cells can have a larger impact on the autoinducer concentration that the cells experience than the density of the cells. The spatial distribution will be more relevant at the early stages of biofilm formation when fewer cells are present, and the autoinducer concentration is approaching crucial levels. Older and thicker biofilms can be well above the threshold concentration[85], rendering the exact spatial distribution, mass-transfer regime and cell density insignificant.

Note that spatial distribution and cell density are independent measures; they are not coupled in such a way that one can be expressed as a function of the other (FIG. 4). The fact that density, spatial distribution and mass-transfer properties are not necessarily coupled can also be demonstrated by varying them independently in experiments. Cell density in liquid cultures can be altered without changing mass-transfer properties or spatial patterns. Mass-transfer rates could be manipulated without changing density or spatial patterns, for example, by culturing cells in a membrane reactor and varying the velocity of the medium flowing over the membrane, thereby varying the rate of autoinducer mass transfer from the culture. It is conceivable to change the spatial pattern of cells without changing their density in an exponentially growing liquid culture of cells that have been engineered to express autoaggregation factors to various degrees, leading to the formation of clusters or clumps of aggregated cells (if the clusters become too large, mass transfer will be reduced).

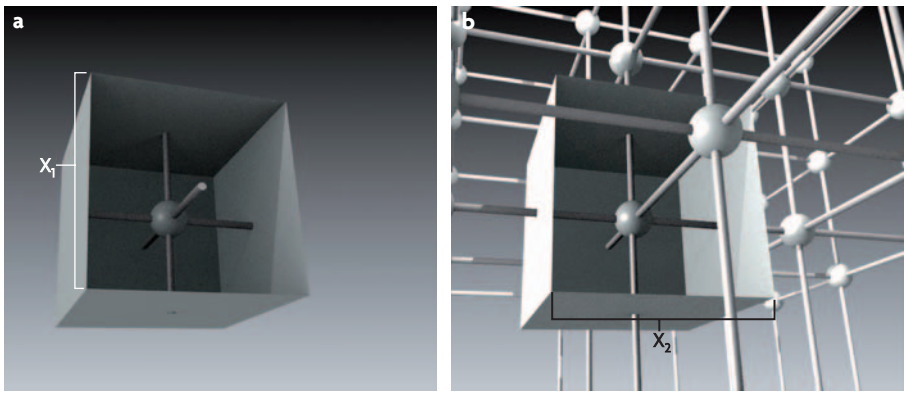


Figure 3 | **The geometric relationship of DS and QS as extreme cases of ES. a** | The concept of diffusion sensing (DS), in which a cell uses the production of a diffusible autoinducer to identify mass-transfer-limited regimes, is shown. This can be exemplified by placing a single cell in the middle of a cube with impermeable walls, which resembles a crevice in a soil particle or a patch of a thin liquid film covering a plant leaf. The size of this confinement is defined by the edge length (x_1) of the cube. **b** | The concept of quorum sensing (QS), in which cells use the production of a diffusible autoinducer to measure their own density, is shown. This can be exemplified by placing a population of cells with a given uniform density at the equidistant nodes of a regular lattice, with infinite extent for simplicity. The bacterial density is inversely proportional to the distance between two neighbouring cells, which is the same as the size (edge length x_2) of an imagined cube with permeable 'walls' surrounding each cell. In both cases, the equilibrium autoinducer concentration depends on the production rate and abiotic decay rate. The cube size x_1, at which the threshold autoinducer concentration is reached in the DS situation, is identical to the cube size x_2 for reaching the threshold concentration in the QS situation. For example, assuming a critical cell density of 10^8 cells ml^{-1} (REFS 7,99), and an equidistant spacing of cells, the mean distance between two cells at the critical cell density, x_1 or equivalently x_2, is 22 μm (10 μm for 10^9 cells ml^{-1}, 46 μm for 10^7 cells ml^{-1}), corresponding to a volume of 10^4 μm^3 (10^3 μm^3 or 10^5 μm^3, respectively) for the box surrounding a cell in (**a**). This is in line with findings of Barak and Ulitzur[100], who noted that the threshold density on solid medium was only approximately 1 cell per 100 μm^2.

Despite the relevance of spatial distribution in natural systems with limited mixing, we do not propose that detecting clustering is the function of autoinducer sensing, as we argue that information the cells cannot obtain independently, such as cell density, spatial distribution or mass-transfer properties (FIG. 1), cannot be the ecologically relevant function of the sensing mechanism. Rather, we propose that it is the combined assessment of cell density, mass-transfer properties and spatial distribution that is ecologically relevant. We develop this into the unifying hypothesis of ES in the next section (TABLE 1).

The ES hypothesis

We propose ES as a unifying concept for bacterial communication that acknowledges the fact that cells, using autoinducers, can only measure the combination of cell density, spatial distribution and limitations to autoinducer mass transfer. The term 'efficiency sensing' conveys the idea that cells produce and release diffusible autoinducer molecules into the environment as a proxy for testing the efficiency of producing costlier diffusible extracellular effectors. If all produced effector molecules were lost, the efficiency would be 0%. If all remained in use, the efficiency would be 100%. Autoinducer concentration can be used as a proxy for effector concentration because both are subject to basically the same combination of influencing factors. Therefore, their concentrations will usually be highly correlated, such that the autoinducer concentration will actually predict the attainable effector concentration better than it will predict the previously proposed functions of sensing, such as cell density and mass-transfer limitations. The concentration of autoinducer, and therefore effector, attainable for a particular cell in a particular location is in fact the aggregated information relevant to the fitness of this cell, not cell density, spatial distribution of the neighbours or rates of mass transfer by diffusion and advection as such.

ES is a cost saver on three accounts. First, a low-molecular-mass compound such as AHL requires less energy and carbon or nitrogen to produce than a macromolecule such as a protein. Second, the amount of autoinducer produced can be kept low if its concentration is measured with high sensitivity, whereas effectors might require higher concentrations. Third, many different effectors might have to be produced,

such as a cocktail of hydrolytic enzymes for the breakdown of polymeric organic material, but only one type of autoinducer molecule might suffice.

The ES concept combines a unifying hypothesis of what cells measure (cell density, mass-transfer properties and spatial distribution) with a unifying hypothesis of why they measure it (to estimate the efficiency of producing extracellular effectors and react accordingly). This function of ES does not, on purpose, stipulate that the extracellular effectors are produced cooperatively because this is not always the case (cells could be alone yet produce autoinducers and effectors). Nevertheless, cooperative and coordinated production of effectors emerges in many situations in which neighbours with the same autoinducer system are present. ES therefore encompasses cooperative and non-cooperative situations. The evolutionary hypothesis that ES evolved because of the direct fitness benefits of optimized effector production for the individual and because of group benefits of cooperative effector production also encompasses QS and DS concepts. As direct and group benefits work in the same direction, ES would evolve under broader conditions than QS and DS. Interestingly, the function of positive feedback might be to speed up the upregulation of cells in the vicinity or at the periphery of a cluster, enabling a synchronized response, even for irregularly located cells.

Note that autoinducer sensing is often connected with switching from one life strategy to another (for example, from non-virulent to virulent), which involves the autoinducer simultaneously upregulating one set of genes and downregulating another[86]. Some of these genes might be involved in effector release and therefore ES, whereas others might not. Therefore, the expression of genes that are not involved in effector release can indirectly depend on ES because of their coupled regulation.

Microcolonies — avoiding problems

Microcolonies are clusters of cells. In soil and many other habitats in which bacteria grow predominantly attached to surfaces, these clusters typically arise from the growth and cell division of a progenitor cell (FIG. 2); they are therefore essentially clonal[45,87–89]. The more the cells are clustered together the sooner the threshold for induction is reached. This, together with the usual positive feedback of autoinducer production, shelters communication within clusters (BOX 1). The fewer and larger these clusters are, the more they will exclude interference from other autoinducer-producing or autoinducer-degrading bacteria or plants and other eukaryotes. Although there is potential for cross-talk among producers of overlapping sets of autoinducers in the rhizosphere[35–38,40–44], the clustered spatial distribution of strains will diminish the opportunities for cross-talk in practice. To the extent that biofilms consist of clonal microcolonies as building blocks, this also holds true for biofilms, but twitching motility or other forms of surface-bound motility can spread motile strains or subpopulations around and over non-motile microcolonies, bringing them into close contact[89].

Clonal microcolonies not only limit interference but also promote the evolution of cooperation by avoiding conflicts of interest, as discussed above. The relatively stable spatial structure of related individuals in a microcolony guarantees that most interactions are with next of kin, in contrast to planktonic bacteria. Also, the lack of mixing promotes coexistence[90], which is in fact the very reason[91] for the extremely high diversity of bacteria in soil[47–50]. Clustered growth therefore promotes diversity between clusters and avoids consequences such as interference and conflicts of interest. Ironically, although autoinducer-based sensing has been studied in depth in well-mixed liquid cultures, the very behaviour studied could not have evolved under these conditions — DS because it does not make sense in bulk liquid and QS because it is not evolutionarily stable when cooperators and cheaters are mixed.

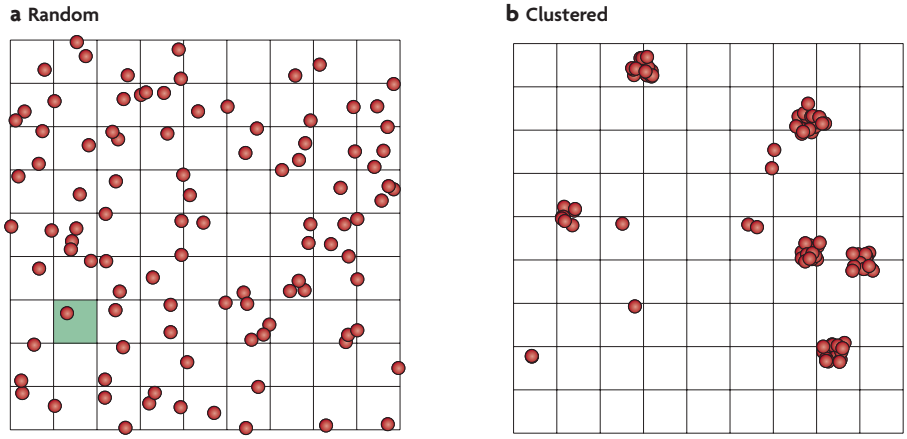


Figure 4 | **Spatial distribution and cell density.** Both panels contain 100 cells in a domain of the same size, so the cell density is the same. **a** | A random spatial distribution of cells (random numbers from a uniform probability distribution, in which all positions are equally likely), is shown. Note that positioning cells randomly will result in some clusters, which are probably not clonal. This situation could represent a snapshot of a mixed liquid culture or cells that disperse after cell division. **b** | Shows a random distribution of clusters that have been growing exponentially at a uniform rate for a randomly chosen time interval (limited to four divisions or 16 cells). Within each cluster, the cells are located randomly in a box of 5% of the domain size. This situation could represent microcolonies founded by single cells in random locations and at random times. Now consider replacing population-scale cell density (the density of cells in liquid culture or in a biofilm) by cellular-scale cell density, defined over a small reference volume, such as the volume elements demarcated by the grid in (**a**) and (**b**). As a consequence, such a small-scale density, for example, the density of 1 cell per unit volume in the green box in (**a**), does not account for the presence or absence of cells in the neighbouring volume elements, which clearly affect the autoinducer concentration in the focal volume element (BOX 1). Even within a cluster, the peripheral cells sense a different autoinducer concentration than the central cells. Therefore, autoinducer sensing in clustered cells cannot be simplified as the measurement of cell density on the small scale of the cluster volume.

Conclusions and future perspectives

QS proposes that cells use autoinducer sensing to measure their density, and DS proposes that they measure the mass-transfer properties of their environment. However, we have shown that the spatial distribution of autoinducer-producing neighbouring cells can have a stronger impact on the autoinducer concentration that a given cell experiences than the cell density. Further, we have shown that a typical feature of autoinducer sensing — positive feedback in autoinducer production — favours upregulation of cells in small clusters and their vicinity.

As the three key determinants of autoinducer concentration (cell density, mass-transfer properties and the spatial distribution of cells) can vary independently,

Glossary

Accounting cost
Accounting cost represents the total amount of money spent on buying or producing something.

Advection
The transport of material with the flow of, for example, water or wind; more generally, the motion of a conserved quantity in a velocity field.

AHL
N-acyl-L-homoserine lactone. A class of small autoinducers produced by a considerable number of Proteobacteria, consisting of a homoserine lactone ring with a *N*-linked acyl side chain of variable length (C_4–C_{20}) and functional groups (mostly hydroxyl- or oxo- groups at the C_3 position).

AI-2
Autoinducer 2. Derived from the methyl-donor S-adenosylmethionine, which forms S-adenosylhomocysteine after the transfer of the methyl group. In some bacteria this is converted to S-ribosylhomocysteine, which is converted by the enzyme LuxS to homocysteine and 4,5-dihydroxy-2,3-pentanedione, which spontaneously cyclizes into several furanones in chemical equilibrium, collectively referred to as AI-2.

Autoinducer
A molecule secreted by a bacterial population that accumulates in the growth medium and induces genes in that same bacterial population.

Cheater
An individual who obtains more benefits from a collectively produced public good relative to its own contribution.

Chemical manipulation
If the chemical is produced for the purpose of changing the behaviour of the receiver but this change is detrimental for the receiver, this is chemical manipulation.

Coincidence detection
A situation involving two independent inputs and an output that is activated only when signals are received at the same time at both inputs.

Communication
True communication or signalling requires that the sender, having incurred costs in signal production, benefits from the response of the receiver and that the receiver in turn benefits from its own costly response to the signal. Otherwise, it is not a signal, but a cue or chemical manipulation.

Cooperation
A proportional contribution by individuals to a collectively produced public good.

Cue
If bacteria use the information from chemicals produced for purposes other than communication, the chemical is not a signal but a cue.

Diffusion
The movement of particles from a higher to lower concentration, in which the net flux of the particles is equal to their diffusivity multiplied by the negative concentration gradient.

Diffusion sensing
Determines whether secreted molecules move rapidly away from the cell, allowing cells to regulate the secretion of degradative enzymes and other effectors to minimize losses owing to extracellular diffusion and mixing.

Direct fitness
The component of fitness that is gained through reproduction in contrast to indirect fitness, which is the component of fitness that is gained from aiding the reproduction of non-descendant relatives.

Effector
A substance that is produced and released into the environment for its ultimate effect, such as exoenzymes, siderophores, antibiotics, biosurfactants and virulence factors. Signals that lead to the production of such compounds are not themselves effectors.

Honesty
An honest signal is one that does not misrepresent the world. If the sender has an incentive to convey wrong information, dishonest signals might evolve that manipulate the receiver's behaviour to benefit the sender.

Interference
A costly activity with a negative effect on the competitor, which does not act indirectly through resources.

Kin discrimination
When behaviours are directed towards individuals depending on their relatedness to the actor.

Kin selection
Favours traits because of their beneficial effects on the fitness of relatives.

Occam's razor
A principle stating that the explanation of any phenomenon should make as few assumptions as possible. When multiple competing theories have equal predictive powers, the principle recommends selecting the explanation that makes the fewest assumptions and postulates the fewest hypothetical entities.

Opportunity cost
Opportunity cost, also referred to as economic cost, is the cost of something in terms of an opportunity forgone. For example, if a city decides to build a hospital on vacant land that it owns, the opportunity cost is the best other thing that could have been done with the land and construction funds instead.

Pheromone
A chemical produced by an organism to transmit a message to other members of the same species, affecting their behaviour or physiology.

Phylloplane
Aerial plant surfaces, such as the stem, leaves and flowers.

Public good
Any fitness-enhancing resource that is accessible to multiple individuals within a local group.

Quorum sensing
Determines whether a sufficient cell density has been reached to switch a set of behaviours of a whole population, and synchronizes this switching among all individuals of the population.

Rhizosphere
The rhizosphere is the root surface (also known as the rhizoplane) and the surrounding soil influenced by plant roots, as opposed to the bulk soil.

Signal
Any act, structure or chemical emission that elicits a response from the receiver and that evolved because of this effect and is effective because the receiver's response has also evolved. Note that the diffusible signals typically used by bacteria blur the distinction between emitter and receiver. In diffusion sensing, the signal will even be received only by the sender. As such 'self-signalling' does not preclude an evolved response, we use signal and signalling in a broader sense in this article that includes 'self-signalling'.

cells sensing the autoinducer concentration are unable to distinguish cell density from mass-transfer properties or spatial distribution. They can only assess the combination of these factors. Therefore, we propose that the ecologically relevant function of autoinducer sensing is to assess the efficiency of producing diffusible extracellular effectors, as their concentration will be influenced by the same factors that influence the concentration of diffusible extracellular autoinducers. The autoinducer can function as a proxy for the more expensive effectors such as exoenzymes. ES unifies the concepts of what cells sense, why cells sense and the evolutionary hypotheses of the fitness benefits derived from autoinducer sensing. ES can be robust towards problems of complexity and cheating *in situ* if it mainly takes place in clonal microcolonies, which can shelter communication within the cluster from cross-talk even in diverse habitats and which can promote the evolution of cooperative behaviour.

We are only beginning to understand the complexities and evolutionary consequences of autoinducer sensing. Progress in this direction will be aided by a more quantitative approach, including measuring the rates of autoinducer and effector production. Competition experiments with mutants that differ in the rates of autoinducer and effector production will elucidate the fitness costs and benefits of bacterial communication. Further, we should shift our attention from pure cultures in well-mixed liquids to conditions that are relevant *in situ* to generate spatial data that can be analysed with the help of mathematical models. Given the importance of communication in the interactions of pathogenic or mutualistic bacteria with their hosts, such an understanding of the fitness effects of communication can guide rational treatment strategies and improvements in agricultural practice.

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doi:10.1038/nrmicro1600

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Acknowledgements

We thank Dieter Haas for discussing the fitness effects of signal-blind and other mutants. We apologize to the authors of the many interesting studies that we could not discuss due to space limitations. We thank the Deutsche Forschungsgemeinschaft (DFG) for financial support through the collaborative research centre on 'Singular Phenomena and Scaling in Mathematical Models' and the GSF - National Research Center for Environment and Health for supporting the project network 'Molecular Interactions in the Rhizosphere'.

Competing interests statement

The authors declare no competing financial interests.

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