Distance-dependent danger responses in bacteria
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The last decade has seen a resurgence in our understanding of the diverse mechanisms that bacteria use to kill one another. We are also beginning to uncover the responses and countermeasures that bacteria use when faced with specific threats or general cues of potential danger from bacterial competitors. In this Perspective, we propose that diverse offensive and defensive responses in bacteria have evolved to offset dangers detected at different distances. Thus, while volatile organic compounds provide bacterial cells with a warning at the greatest distance, diffusible compounds like antibiotics or contact mediated killing systems, indicate a more pressing danger warranting highly-specific responses. In the competitive environments in which bacteria live, it is crucial that cells are able to detect real or potential dangers from other cells. By utilizing mechanisms of detection that can infer the distance from danger, bacteria can fine-tune aggressive interactions so that they can optimally respond to threats occurring with distinct levels of risk.

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Introduction
New methods in imaging and genome sequencing have reaffirmed and expanded our appreciation of the diversity of bacterial communities in nature [1–3]. However, as powerful as these techniques are, they serve mainly to catalogue bacterial diversity while offering limited insights into the behaviors of the constituent communities. Are coexisting bacteria competing with one another or cooperating for their mutual benefit? Over the last few decades the pendulum on these questions has swung fairly broadly in both directions, and has led to productive and valuable research enterprises across both extremes [4,5]. Cooperative interactions mediated by, for example, cross-feeding or quorum sensing, are widespread, and can alter bacterial behaviors for a variety of traits linked to bacterial fitness [6–9]. At the same time, surveys from natural populations have found that while cooperative interactions between bacteria exist, they are far less common than competitive interactions [10]. Indeed, the last 10 years has seen a renaissance in identifying and understanding the diverse means by which bacteria compete and kill one another. Antagonism is rife and is coordinated by a growing arsenal, including antibiotics, bacteriocins, volatile organic compounds (VOCs), and different forms of contact-dependent killing. But why have bacteria evolved so many ways to damage one another? Using results mainly based on studies of bacterial co-cultures, we hypothesize that these diverse mechanisms of antagonism have evolved as non-redundant responses to threats occurring at different distances from a focal cell.

Distance-dependent danger sensing
Bacteria need to be able to detect and discriminate between different kinds of biotic threats in their immediate environment. However, because these threats occur at different spatial scales, they also call for different types of responses. Recently, Cornforth and Foster proposed the idea of Competition Sensing whereby bacterial cells respond to the direct harm caused by competing cells or to nutrient limitation [11]. Similarly, LeRoux et al. proposed that bacteria detect ecological competition by sensing danger cues of competition, rather than direct harm per se. Such cues can include material from lysed kin cells or diffusible signals from competitors that are detected by a dedicated danger sensing signal transduction mechanism that activates a danger response regulon [12]. Both ideas are important because they make clear that bacteria integrate features of the biotic environment via cues before eliciting a potentially metabolically costly response [11,13]. However, it is also important to determine if the nature of these cues directs the form of the response. Our review of the literature suggests that it does (Tables 1 and S1). We consider three broad categories of cues (Figure 1) that are detected at decreasing distances and which indicate different levels of danger: VOCs, diffusible compounds, and those that are contact-dependent. Although these categories are admittedly arbitrary and occasionally overlap, they help to classify examples where these distinct cues induce different types of offensive or defensive responses in target organisms. We consider caveats and limitations with this classification and questions for future studies below.
Volatile organic compounds

VOCs are low molecular weight compounds (<300 Da) that can readily evaporate at ambient temperatures and air pressures [14,15]. Because of these properties volatiles can disperse through both water- and gas-filled pores in the soil, making them extremely suitable for long distance interactions in these spatially complex environments. Volatiles are often considered to be side products of primary metabolism, but this viewpoint is challenged by findings that many volatiles demonstrate biological activity [16], such as antibacterial or antifungal activity [17,18**]. Volatile blends differ among bacterial species, thereby raising the possibility that these long-distance cues can inform other species of the specific identity of the producers [19]. At the same time, because VOCs can travel far from their source of production, their detection at low concentrations implies that possible threats from these species, due potentially to the direct antimicrobial effects of the VOCs themselves [20,21], are not imminent. Accordingly, and given their diverse chemistries, we predict that detection of microbial VOCs will lead to generalized mechanisms of defence. These include different forms of escape together with the induction of more broadly effective modes of protection. Growth, motility and biofilm formation can all be modified by VOCs at low concentrations (Table 1), as can the

**Table 1**

| Category           | Sub-category                              | Growth         | Antibiotic resistance | Motility       | Biofilm formation | Antibiotic production | T6SS      |
|--------------------|-------------------------------------------|----------------|-----------------------|----------------|-------------------|-----------------------|-----------|
| Volatiles          | Volatile blend                            | [19,21]        | [23,26,57]            | [57]           | [20,57,59]        | [20,59–61]            | [19]      |
|                    | Volatile compounds                       | [17,20,22]     | [20,24,26,27,57,58]   | [20,57,59]     | [20,59–61]        | [20,59–61]            | [19]      |
| Diffusible molecules | Diffusibles produced by other bacteria    |                |                       |                | [13*,25,26,28,29] |                       |           |
|                    | GlcNAc or peptidoglycan (Sub-MIC) antibiotics |                |                       |                | [31,40]           |                       |           |
|                    | Quorum sensing molecules                  |                |                       | [62]           | [62,63]           | [32,33,37]           | [53,63]  |
|                    | Kin cell lysis                            |                |                       | [25]           | [25]              |                       | [51]      |
| Contact            | CDI                                       | [64,65]        | [45]                  | [45]           | [48*,49,51]       | [49,51]              |           |
|                    | Type VI SS                                |                |                       | [48*]          | [51]              |                       |           |
|                    | Type VI SS toxins                         |                |                       |                | [32]              |                       |           |
|                    | Type VI SS                                |                |                       |                | [32]              |                       |           |
|                    | Type VI SS and Type VI SS induced lysis of kin cells | [51] | | | | | |

**Figure 1**

Distance-dependent danger sensing in bacteria. Soil is a spatially heterogeneous environment consisting of soil particles, shown in grey, and water-filled and air-filled pockets, shown in white. Because of these physicochemical properties volatiles (shown in blue) can diffuse over long distances. Sensing volatiles provides information about the presence of a distant competitor and induces protective responses including an increase in antibiotic resistance. At a closer range diffusible molecules (shown in red), for example, antibiotics, signal the presence of a competitor in the near vicinity, which requires a counterattack such as the induction of antibiotic production. Cell-contact mediated antagonism such as a Type VI secretion system (T6SS) attack (shown in purple), invokes an immediate T6SS counterattack. Responding cells in all panels are shown in orange.
induction of developmental transitions in microbial colonies. For example, trimethylamine produced by *Streptomyces venezuelae* induces the production of a novel cell type in other streptomycetes, called explorers, that rapidly disperse away from high levels of local competition and towards higher resource concentrations [22]. In addition, bacteria consistently respond to VOCs by increasing antibiotic resistance, even if the volatiles themselves have no antimicrobial properties. For example, *Escherichia coli* increases its resistance to gentamicin and kanamycin after exposure to *Burkholderia ambifaria* volatiles [23]. *Pseudomonas putida* reacts to indole produced by *E. coli* by inducing an efflux pump that increases resistance to several antibiotics [24]. Importantly, *P. putida* cannot produce indole itself, providing direct evidence that bacteria can alter their intrinsic levels of antibiotic resistance in response to volatile bacterial cues. Similarly, *Acinetobacter baumannii* responds to the *P. aeruginosa*-produced small volatile 2’ amino-acetophenone (2-AA) by altering cell-wide translational capacity and thereby increasing the production of antibiotic-recalcitrant persistor cells [25]. Although these results are suggestive, it is important for future studies to distinguish the direct influence of VOCs on cells from their indirect effects mediated by the changes they induce in the test environment. For example, ammonia and trimethylamine, volatiles produced by *E. coli*, appear to increase tetracycline resistance in both Gram-positive and Gram-negative bacteria, while these volatiles did not display any growth toxicity at the same concentration [20]. However, rather than directly inducing a response in a target cell, the result was instead explained by the effects of these VOCs on environmental pH; this change, in turn, lead to reduced antibiotic transport [20,26] and therefore increase resistance. Similarly, VOC-mediated modifications to environmental pH may permit cells to grow at higher antibiotic concentrations because low pH can inactivate the antibiotic [27]. Although more work is needed to identify the mechanisms underlying many of the changes elicited by volatiles, studies thus far suggest that these compounds induce protective responses.

**Diffusible molecules**

Bacteria produce a vast diversity of diffusible compounds as products of primary and secondary metabolism. While some, like quorum-sensing molecules, tend to bind targets within species to induce cooperative responses (although cross-species induction has been observed) [28], many others are antagonistic, for example, antibiotics or bacteriocins. Additionally, because diffusible molecules will often mediate their effects at shorter distances from their producer than volatiles, their detection will indicate that a potential competitor may be nearby. Many recent studies (Table 1) have shown that bacteria modify their metabolome and their antimicrobial activity when co-cultured with or in close physical proximity to competitors [13*,29–33]. Indeed, because of this, such co-cultures offer promising avenues for drug discovery [34]. When the Gram-positive actinomycete *Streptomyces coelicolor* was co-cultured with other actinomycetes [30] or with fungi [35] it produced many compounds, including secondary metabolites and siderophores, that were not detected in monoculture, and which were often unique to a specific interaction. Similarly, the inhibitory range of individual streptomycete species increased by more than twofold during bacterial co-culture [13*]; the distance-dependence of these responses is consistent with the idea that induction was coordinated by diffusible molecules and not VOCs (unpublished results). Notably, antibiotic suppression is also observed during these interactions [13*,29,36], highlighting that the cells producing diffusible molecules can also strongly influence the outcome of pairwise interactions.

While studies between co-cultured cells provide insights into the dynamics of competition mediated by diffusible molecules and show how widespread these responses are among different phyla [29], they do not always reveal the types of diffusible molecules that mediate these effects. For this reason, it has been valuable to focus on model species, and these too have shown that secreted antibiotics at inhibitory and sub-inhibitory concentrations can induce well-known secondary metabolite pathways [32,33]. For example, co-cultivation of *S. venezuelae* and *S. coelicolor* induced undecylprodigiosin production in the latter while also stimulating its morphological differentiation [37]. This response was induced by the angucycline antibiotic jadomycin B, produced by *S. venezuelae*, which binds the “pseudo” gamma-butyrolactone receptor SchbR2 in *S. coelicolor* and thereby directly regulates these two processes. The fact that angucyclines from other streptomycetes can also bind this receptor suggests that induction by this diffusible molecule is likely to be widespread [37]. A related study in these same species revealed that the gamma-butyrolactones, diffusible quorum sensing signalling molecules that activate antibiotic production, could also coordinate bacterial antagonism, because the same molecule regulates antibiotic production in both species [38]; accordingly, if this molecule is produced by one species, it will necessarily induce antibiotic production in the other. In another particularly elegant study, *Vibrio cholerae* was found to change its motility in response to sub-lethal concentrations of the antibiotic andrimid, produced by another *Vibrio* sp., by increasing its swimming speed, turning rate, and run lengths while directing its movement away from the source of the antibiotic [39]. While responding to antibiotics is predicted because these cause direct harm, bacteria can also respond to the products that result from intercellular antagonism. For example, peptidoglycan from the cell walls of Gram-positive bacteria induced the production of the antibiotic pyocyanin in *Pseudomonas aeruginosa* through detection of its monomer GlcNAc [31]. Similarly, cell-wall derived GlcNAc potentially derived
from competing microorganisms can activate antibiotic production in streptomycetes [40]. Like antibiotics, these products of aggression are indicative of imminent danger.

**Direct contact**

At the shortest distance between cells, bacterial antagonism can be mediated by cell–cell contact. Bacteria possess several ways to inhibit other cells through cell contact, such as contact-dependent inhibition (CDI) [41] or Type VI Secretion System (T6SS) [42]. CDI systems, that deliver toxins into target cells, are widespread among Gram-negative bacteria [43]. These systems are composed of a protein with a C-terminal toxic region, an outer membrane transporter for its secretion and an immunity protein [44]. The toxin protein is predicted to extend from the cell surface and upon recognizing a receptor on a target cell, it delivers its C-terminal domain to the target cell where it exerts toxicity [44]. These toxins kill or inhibit susceptible cells lacking immunity, but not sister cells that express cognate immunity. Although sister cells are not killed by the toxin, *Bhurkhorderia thailandensis* cells still respond to attacks by down-regulating their *cdt* operon and, interestingly, by increasing biofilm formation and the upregulation of T6SS and non-ribosomal peptide/polyketide synthase genes [45,46]; these responses can be perceived as forms of defence and offense, respectively. As yet, the molecular mechanism behind this response is yet unknown.

Approximately one quarter of all Gram-negative bacteria possess genes encoding T6SS [47]. The T6SS is a contractile nanomachine resembling a phage tail that translocates toxic effector proteins into a target cell [42]. While some bacteria use their T6SS as an offensive weapon, others use it defensively in response to a T6SS-mediated attack [48]. The best-studied organism in the latter case is *P. aeruginosa*, which does not use its T6SS until it is attacked itself, whereupon it initiates a counterattack. Three different mechanisms through which *P. aeruginosa* can sense an incoming attack have been described, of which two depend on direct contact. *P. aeruginosa* engages in so-called “T6SS duelling” where T6SS-mediated killing activity is regulated by a signal that corresponds to detection of the point of attack by the T6SS of another cell [48,49,50]. In this way the *P. aeruginosa* counterattack is directed precisely with both spatial and temporal accuracy [48]. T6SS duelling was first observed among *P. aeruginosa* sister cells, although this does not result in killing as cells are immune to their own toxins [49]. A T6SS expressing strain of *Agrobacterium tumefaciens* could induce a counterattack by *P. aeruginosa*, but this required the injection of toxins [51]. Finally, *P. aeruginosa* can react to a T6SS attack without being attacked itself in a response known as “PARA” or *P. aeruginosa* Response to Antagonism [51]. In this case T6SS activity is stimulated by the effects of T6SS of a competitor, as these cause kin cell lysis which in turn acts as a diffusible danger signal (cue) that activates their own T6SS. Interestingly, the Type IV secretion system (T4SS), another class of secretion system used for the transport of DNA or proteins [52], can also induce a T6SS counterattack [51,53]. This has been speculated to occur through the sensing of membrane perturbations caused by the incoming nanomachine [53], or through T4SS mediated lysis of kin cells that induces the PARA response [51]. Although this research area is biased to few species (e.g., *P. aeruginosa* and *Serratia marcescens* [54,55]) responses to T6SS attack appear to be limited to T6SS-mediated counterattack and show that when threats are detected at close range, offensive counterattack is the anticipated response.

**A broader perspective on distance-dependent danger responses**

Ecological competition is typically partitioned into two broad types: resource competition and interference competition [11]. While studies over several decades have uncovered the exceptional sensitivity of bacteria to small changes in resource concentrations, we are only just beginning to explore the sensitivity of bacteria to threats from other microbial species. We propose that the concentration of volatile compounds, diffusible molecules, and direct and indirect effects of cell-contact provides information about the distance of cells from the producers of these molecules and that these direct how bacteria respond to them. This view is supported by the studies we examine as well as the vast literature on the response of bacteria to sub-MIC antibiotic concentrations (Tables 1 and S1). But these limited studies suffer from some important limitations. First, the current literature is highly biased with respect to organism and response. Pathogens are overemphasized because of our justified concerns with how these species will respond to suboptimal drug dosing, while resistance is favoured for the same reasons. Other modes of defence may be more widespread; however, these remain to be fully explored. Second, while our categories are useful, they are also both arbitrary and coarse, as “distance” and its detection are likely to be both environment and species specific. For example, in heterogeneous soil environments, the distance that diffusible or volatile compounds travel depends not only on the actual distance but also on the presence or absence of water or air filled pockets as well as on the temperature. Moreover, to distinguish between these threats from different distances, bacteria need to be able to differentiate between volatile and diffusible compounds across a range of concentrations. The molecular mechanisms underlying how these compounds are detected are not yet well understood. Third, our selection of examples is fragmented and potentially biased towards responses that match our expectations, however unintentionally. Finally, at present we lack a broader mechanistic or theoretical framework in which to examine these responses, both from the perspective of the cells producing danger cues as well those responding
to them. These latter issues, in particular, suggest many questions that are important to consider as we move forward. Most importantly, how can cells distinguish true threats from marginal ones, or even cues from mutualistic bacteria, so that they can avoid paying the costs of a misfired response? Indeed, what are the costs of misfiring? This is particularly important to consider if danger cues are durable and persist long after they were first produced. In addition, although we focus on how cells respond to different cues, it is equally crucial to consider why and when these cues are produced in the first place. At least for antibiotics, evidence suggests that these secondary metabolites are used as weapons and not signals [13]. However, this still leaves open the question of whether these cues, or weapons responding to the threat of harm, are mainly used for offense or defense. Similar questions remain for VOCs that are variously considered as weapons or signals for inter-species and intra-species communication [56]. Addressing these issues from the perspective of the producer of VOCs, diffusible compounds, and contact-dependent weapons will undoubtedly illuminate our understanding of how bacteria respond to these cues of danger in their natural environments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mib.2017.02.002.

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