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Review

The Evolution and Ecology of Bacterial Warfare

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Bacteria have evolved a wide range of mechanisms to harm and kill their competitors, including chemical, mechanical and biological weapons. Here we review the incredible diversity of bacterial weapon systems, which comprise antibiotics, toxic proteins, mechanical weapons that stab and pierce, viruses, and more. The evolution of bacterial weapons is shaped by many factors, including cell density and nutrient abundance, and how strains are arranged in space. Bacteria also employ a diverse range of combat behaviours, including pre-emptive attacks, suicidal attacks, and reciprocation (tit-for-tat). However, why bacteria carry so many weapons, and why they are so often used, remains poorly understood. By comparison with animals, we argue that the way that bacteria live — often in dense and genetically diverse communities — is likely to be key to their aggression as it encourages them to dig in and fight alongside their clonemates. The intensity of bacterial aggression is such that it can strongly affect communities, via complex coevolutionary and eco-evolutionary dynamics, which influence species over space and time. Bacterial warfare is a fascinating topic for ecology and evolution, as well as one of increasing relevance. Understanding how bacteria win wars is important for the goal of manipulating the human microbiome and other important microbial systems.

Introduction

Bacteria commonly live in dense, multispecies communities where there is competition over scarce resources. A key requirement for evolutionary success in these complex environments is the ability to survive and divide in the presence of other strains and species [1–4]. As a result, metabolism and the ability to acquire nutrients are key determinants of success in a given community [5]. For example, mammalian gut bacteria employ a wide range of enzymes and transporters to break down and import carbohydrates [6]. Such mechanisms of **exploitative competition**, however, are only part of the story. It is becoming increasingly clear that bacteria also rely heavily on mechanisms of **interference competition** for their ecological and evolutionary success [7–14]. (See the Glossary in Box 1 for key terms denoted in bold text.)

Advances in genomics, biochemistry and imaging have revealed that bacteria employ an amazing diversity of mechanisms to harm, inhibit and kill off their competitors (Figures 1–3). These bacterial **weapons** include mechanisms for chemical warfare via **toxins**, complex mechanical weapons that punch holes, and the use of viruses in biological warfare [15]. Deployment of these weapons can be extremely costly; at the extreme, a cell may go so far as to lyse and die to attack others. And yet, many species carry multiple types of weapons and sometimes multiple variants of each type (Figures 2 and 3). It is not yet clear what drove the evolution of all of these bacterial weapons. This is perhaps surprising insofar as the 1928 discovery of the antibiotic penicillin marked the starting point of close to a century of intense investigation of antimicrobial compounds, many of which first evolved as mechanisms for microbial **competition**. However, the focus on the drugs' mechanisms of action and clinical application has meant that a literature replete with examples of antibacterial mechanisms rarely considers their evolution or original function [16].

Outside of microbiology, the evolution of **combat** has been long discussed, following the classic paper of Maynard Smith and Price [17] that introduced **game theory** into evolutionary biology. However, the focus was animal combat and more specifically why animals that have weapons rarely use them in pairwise **contests** [17–19]. This focus contrasts with what we know about bacterial contests, where encounters can involve many millions of individuals on each side and, moreover, contests are often intense and lethal [5,16]. The evolution of **warfare** in bacteria, therefore, demands dedicated study. In the years following the development of evolutionary game theory, some authors employed theory and experiment to ask when bacterial weapons are favoured [20–26] and how weapons can affect genetic diversity [27–33]. Most recently, some studies have begun to consider the evolution of the behavioural strategies used when bacteria fight, including responses to nutrient levels [16], reciprocation [34] and provocation [35]. Nevertheless, there is much to learn. In particular, we do not understand why bacteria appear to be so very aggressive, both in terms of how often they attack other strains and the amount of weaponry they carry.

Here we showcase the diverse mechanisms that bacteria have evolved to harm and kill their competitors. We discuss both the evolution of the weapons themselves and the evolution of the strategies employed during contests, where we draw comparisons to the much-studied contests of animals. Finally, we turn to the consequences of such prevalent **aggression** for the ecological and evolutionary dynamics of bacterial communities. Bacterial warfare encompasses many major themes in evolution and ecology, including collective behaviour, behavioural ecology, social evolution and the study of ecological networks. Understanding how bacteria win their contests also has growing importance for microbiology and medicine, where many disease

Box 1. Glossary

Aggression	How often and how severely an individual attacks.
Coevolution	Reciprocal evolutionary adaptations in different individuals or species that evolved in response to one another.
Collective behaviour	Behaviour that emerges from the interactions of individuals in a group.
Combat	Fighting involving weapons between two or more individuals.
Competition (as an evolved adaptation [158])	Negative effect of one cell on other cells' survival and reproduction, which evolved at least in part because of this effect.
Competition sensing	A physiological response that detects harm caused by other cells and that evolved, at least in part, for that purpose.
Contest	A fight or battle between two or more individuals.
Cue	A feature that provides another organism with information but that has not evolved for that purpose.
Division of labour	The division of a collective phenotype into separate tasks performed by different individuals.
Exploitative competition	Competition driven by increased uptake and use of nutrients by a focal cell.
Game theory	Theory seeking best performing strategies, where the best strategy will often depend on the strategy of others.
Interference competition	Competition that interferes with the access of other cells to resources, but not driven by increased nutrient uptake in a focal cell.
Quorum sensing	Density-dependent response that occurs via the secretion and detection of dedicated molecules (autoinducers).
Signal	An evolved means of conveying information to receivers (often restricted to cooperative information only [139]).
Strategy	Rule(s) defining the decisions of an actor in response to prevailing conditions (for example, fight back if attacked).
Tactics	Behaviours displayed in a given contest (the realised output of a strategy).
Toxin	Substance that disrupts cell physiology.
Warfare	Conflict involving weapons between two or more groups.
Weapon	Competitive phenotype that damages and/or disrupts the physiology of recipients, and that evolved, at least in part, for that purpose (for example, bacteriocin production).

outcomes rest upon whether a strain can invade or persist in the diverse human microbiota.

The Diversity of Bacterial Weapons

Weapons are extremely common in culturable bacteria (Figure 2). In the best-studied species, it is typical to find multiple mechanisms for damaging competitors, and, moreover, multiple variants of each of these mechanisms (Figure 3). A striking example is the opportunistic pathogen *Pseudomonas aeruginosa*, which is a major concern owing to its ability to cause diverse infections and withstand many antibiotics. A single cell of *P. aeruginosa* has the option of generating a vast arsenal including: many different types of diffusing protein toxins (including S pyocins) [36,37]; multiple types of poisoned molecular speargun (type VI secretion systems) [38]; poisoned proteinaceous sticks (contact-dependent growth inhibition system) [39]; two different mechanical weapons that punch holes in other cells (R and F pyocins) [36]; and viruses (phages) that kill non-clonemates [40,41]. And, though it is less clear they evolved for this purpose, *P. aeruginosa* also makes molecules like hydrogen cyanide [42] and pyocyanin [43], and membrane vesicles [44] that can harm other bacteria. *P. aeruginosa* may be a relatively extreme case, but the widespread occurrence of weapons in the best-studied species (Figure 3) as well as in other cultured bacteria (Figure 2) suggest that, when we look, we will commonly find weaponry in bacteria.

The full extent of bacterial weapons, however, remains unknown. To demonstrate that a protein or pathway functions in interbacterial competition requires culturing and experimental work, which has only been performed for a tiny fraction of bacterial species. Moreover, this fraction is biased as the best-studied species are often pathogens that only cover a minority of the bacterial phylogeny (Figure S1). Pathogens appear to be particularly prone to weapon evolution [45], and it is possible that some unstudied groups may differ so fundamentally in their ecology that the evolution of weaponry is much more limited. For example, the Candidate Phyla Radiation is a fascinating and enigmatic group of ultra-small bacteria that may comprise around a quarter of all bacterial diversity [46]. They have reduced genomes and are thought to live as symbiotic parasites on the outside of larger bacteria [47], and whether they need or use weaponry is not yet clear. Caveats aside, it is clear that many bacteria do employ weapons and, moreover, that these weapons are important for their ability to invade and compete in natural communities [12,13,48–51], particularly in the mammalian microbiome where much attention has been focussed [9,10,12,48,50–57].

Bacterial weapons can be divided up broadly by the way they damage cells (Figure 1). There are some mechanical weapons, which physically damage cells. However, more common are biological (virus) and particularly chemical (toxin) warfare, which

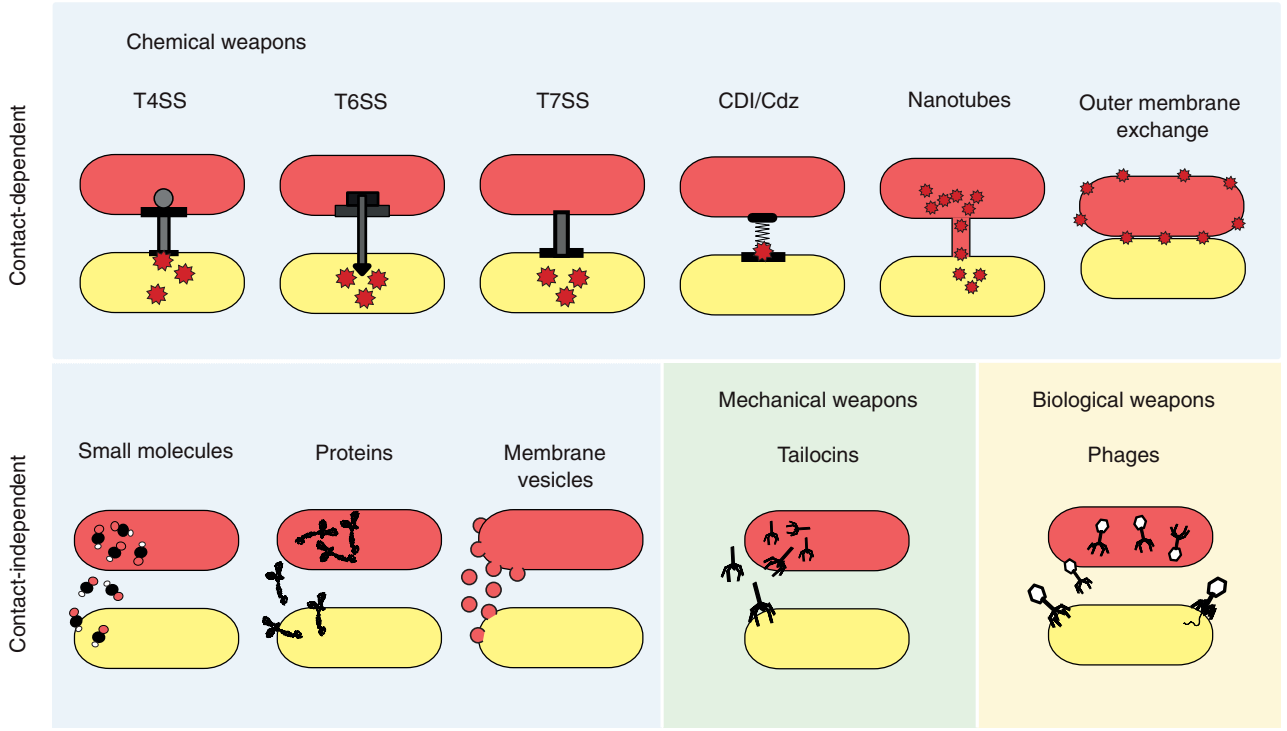

Figure 1. Types of bacterial weapons.
Type IV secretion systems (*T4SS*) translocate DNA and proteins in Gram-negative bacteria and have recently been implicated in targeting toxins towards competitors. *Type VI secretion systems* (*T6SS*) are widespread in Gram-negative bacteria and allow direct delivery of toxin effectors to competitor cells using a repurposed contractile phage tail. *Type VII secretion systems* (*T7SS*) are involved in contact-dependent toxin delivery in Gram-positive bacteria, although little is yet known about their structure and mechanism of delivery. *Contact-dependent growth inhibition* (*CDI*) *systems* (and the functionally similar Cdz systems) involve a filamentous protein containing a toxic domain and a second protein responsible for export and anchoring to the cell surface of the attacker. Upon contact of the filament to a target cell, the toxic domain is translocated into the victim. Cdz toxicity also involves formation of filaments on the cell surface of the attacker. *Nanotubes* are structures bridging the cytoplasm of neighbouring bacteria, allowing the direct transfer of toxins and other molecules between cells. *Outer membrane exchange* (*OME*) mechanisms involve the delivery of toxic proteins embedded in the outer membrane. A cell can poison non-immune neighbours by transferring toxin-containing membrane fragments upon cell–cell contact. *Small molecule toxins* include peptides and antibiotics, less than 10 kDa in size, that are released and diffuse to target cells. *Protein toxins* are those bigger than 10 kDa that are released, often by cell lysis, allowing them to diffuse to target cells. *Membrane vesicles* are produced by diverse bacteria [69] and can kill other cells by, for example, delivery of enzymes that digest the cell wall [44]. The vesicles deliver many molecules, however, and the importance of vesicle production for bacterial competition needs further verification (they do not yet meet the inclusion criteria for Figure 2). *Tailocins* are derived from phages and lack the nucleic acid containing capsular head. These multi-protein assemblies are released into the environment and physically puncture the membrane of their target. *Phages* include many viruses integrated into bacterial genomes that when released will kill competitors but not clonemates that also carry the virus. See main text for more details and Table S1 for references.

dominates the current list of examples. This dominance may partially reflect the historical focus of research on antibiotics, but it is nevertheless clear that toxin-based interference competition is common to many bacteria.

Chemical Warfare

Bacteria use diverse toxins to disrupt the physiology of target cells, ranging from small molecules to large proteins and other molecules. Small toxins often bind to other molecules and interfere with their function [58], whereas protein toxins can have more complex actions, and include large enzymes that digest cell components [59]. Like clinical antibiotics, bacterial toxins typically act on a cell's envelope or its core metabolism. Many toxins made by bacteria damage membranes, including last-line polymyxin antibiotics like colistin [60]. More sophisticated are protein toxins that insert themselves into the target cell membrane and form a pore [61]. These pores weaken or ablate the chemical gradients needed to keep a cell functioning, often leading to cell death. Another major target in the envelope is the cell wall, and its associated protein cytoskeleton, which is again affected by a range of toxins, including beta-lactams and other classes of antibiotics [58]. The last-line antibiotic vancomycin, for example, is made by the soil bacterium *Amycolatopsis orientalis* and destabilizes the cell wall of susceptible cells, leading to death by osmotic lysis [58,62]. Inside the cell, major targets include the transcription and translation machineries, as well as a competitor's DNA and RNA, which are targeted by nuclease toxins [49,61,63].

Regardless of the target, the impact of many toxins rests upon interfering with the cell's ability to grow and divide. The potency of antimicrobial toxins for growing cells makes it imperative that the producer cell has a way to avoid self-intoxication, which is achieved via mechanisms including the use of immunity proteins that block a toxin's activity [61,64], expressing a resistant form of

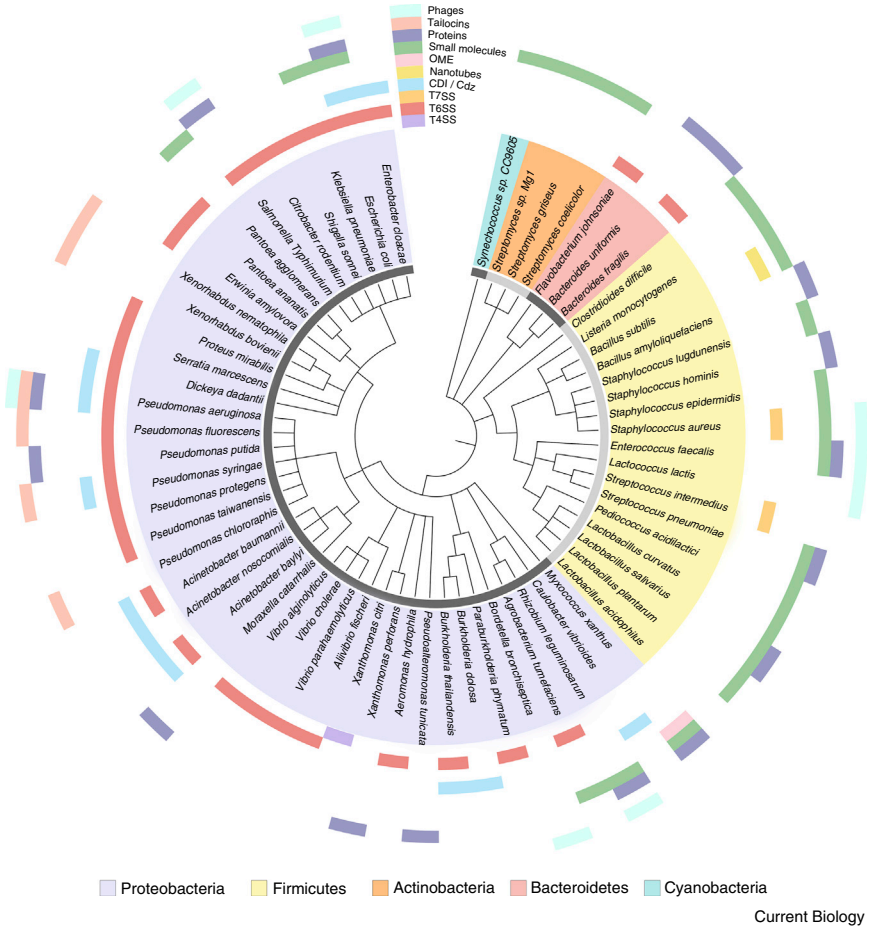

Figure 2. Distribution of documented weapon systems in bacteria.
We performed a literature search to identify studies that provide empirical evidence of a weapon in a given species. Our search used multiple sources and strategies to identify candidates and we then applied fairly stringent criteria for inclusion in the figure (see Table S1 for studies included). Specifically, we required that two strains of the same genetic background were compared with and without the putative weapon system (or toxin of the system) using a competition experiment, which either pitted the two strains against each other or against a third-party strain. We included studies when the presence of the putative weapon resulted in removal and/or inhibition of the competitor in the mixed culture experiment. In a few cases included, the regulation of the weapon needed to be artificially induced to elicit a phenotype. Although many species shown here have multiple weapons, our stringent inclusion criteria mean that many will actually have further weapons that have yet to be validated (for example, prophages). Moreover, the great majority of bacterial diversity is missing as most bacterial species have not yet been cultured, let alone studied in this context (Figure S1). The bacterial phylogeny was built using the NCBI common tree tool [214]. Visualisation and annotation of the tree was performed using iTOL [215].

the toxin's target, synthesizing an inactive precursor, or exporting the toxin to prevent its build-up [65,66]. A catch is that a weapon may be rendered ineffective if a target strain picks up these mechanisms by horizontal gene transfer (see "Ecological and Evolutionary Dynamics" below). Why have bacteria evolved to primarily attack core cellular functions of their victims, like cell wall and DNA synthesis? Most obviously, core functions are often essential for cell viability. Targeting a core function also brings with it the potential to harm a diverse set of competing species. However, to be effective a toxin must be able to reach its target. For this purpose, bacteria have evolved ingenious, and sometimes spectacular, methods of toxin delivery, which we discuss next.

Toxin Delivery: Secretion, Suicide and Stabbing

To employ a chemical attack, a cell must first export its toxins. Small toxins may diffuse out of the producing cell, often via protein pores, or are released by active transport across the cell envelope [67,68]. Other toxins are released in membrane vesicles that bleb from the surface of the producing cell and can deliver many molecules to a target cell in one go [44,69]. At the extreme, several well-known bacteria — including *Escherichia coli*, *P. aeruginosa* and *Salmonella enterica* — have been shown to lyse themselves using dedicated enzymes to release large protein toxins [36,61], thus benefitting their non-lysing clonemates; a particularly clear example of **division of labour** in microbes (below) [34].

Most toxins must cross the membranes of the target cell to have an effect. Again, small molecules may simply diffuse across, and this property has made them particularly attractive for use as clinical antibiotics as they can enter and affect a variety of species [67]. Larger molecules require more sophisticated mechanisms to enter a cell. One elaborate and well-studied mechanism is employed by the colicin toxins of *E. coli*. These large proteins employ a Trojan horse strategy by binding specifically to outer-membrane receptors normally used to import small essential molecules such as vitamin B12, iron-carrying siderophores, or nucleosides. The colicin toxins are massive compared to the intended molecules but, astonishingly, colicins are able to both bind the same receptors and translocate into the cell, either directly or by recruiting host porins [70,71]. The complexity of this entry mechanism, however, makes these toxins highly specific; they fail to bind and enter bacterial species lacking the specific target proteins needed for entry [61].

Whereas many toxins must diffuse from producer to target cell, others are delivered directly to the target. Physical delivery includes direct transfer of membrane components containing toxins when cells touch [72] and the enigmatic formation of 'nanotubes' that appear able to exchange cytoplasmic content, including toxins [73]. Better understood is the appropriately named contact-dependent growth inhibition (CDI) system [74–76]. Something akin to a shape-shifting poison-tipped stick, CDI involves the expression of a filamentous protein, tens of nanometers long, from the cell [77]. Upon contact with the target cell, the toxic domain is delivered to the tip, cleaved [78], and translocated into the target cell [79]. As for protein bacteriocins,

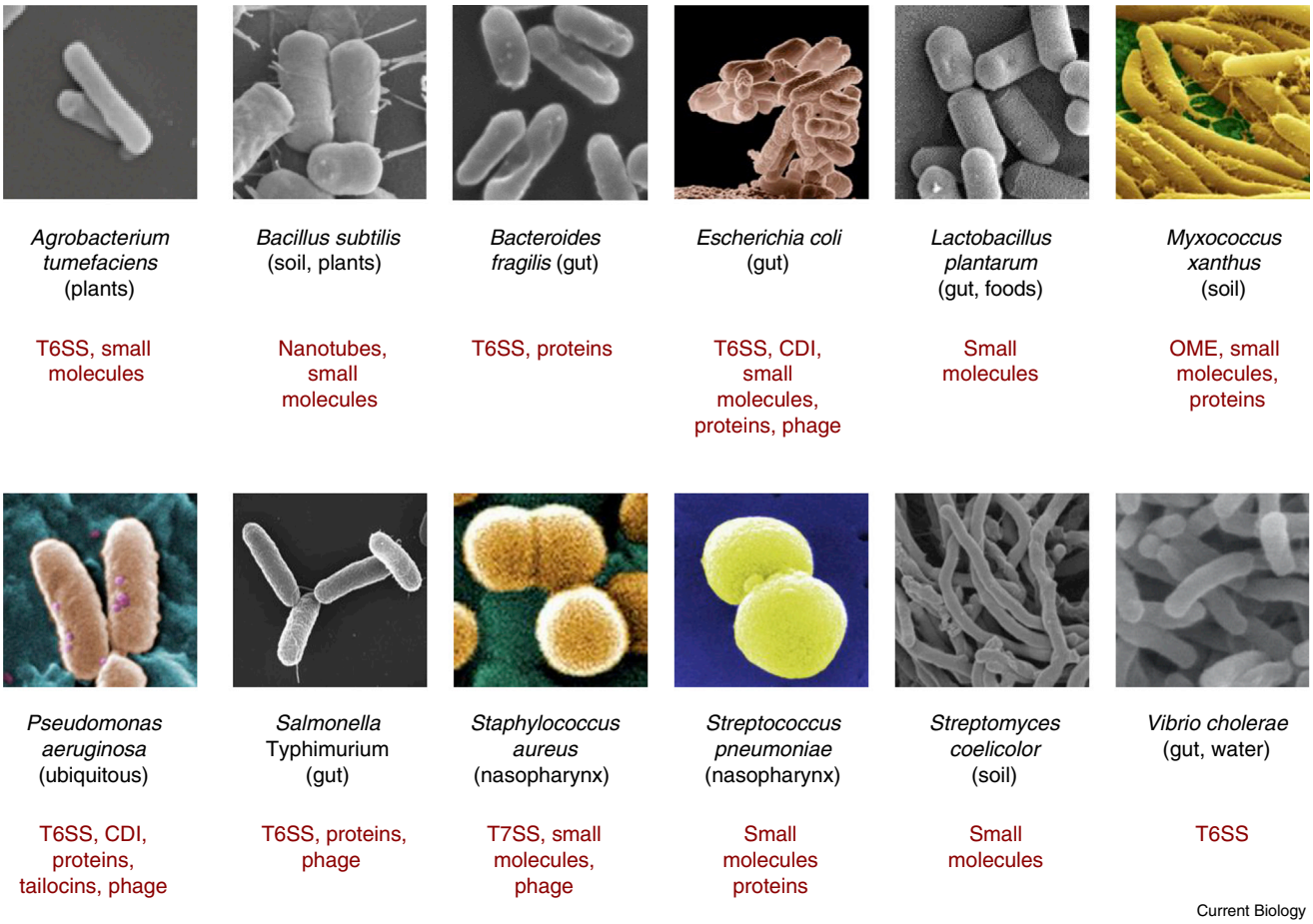

Figure 3. Weapons of well-studied bacteria.
A typical environment for each bacterium is shown in parentheses. Gut and nasopharynx colonization refers primarily to humans, although all of the species can be found in additional environments to those listed. Weapons listed are only those that have been experimentally shown to mediate bacterial competition as described in Figure 2. See Figure 1 legend for details on weapons and abbreviations. Image sources: *A. tumefaciens* (credit: taken from [216] © 2013 John Wiley & Sons Ltd); *B. fragilis* (credit: taken from [217] (CC BY 4.0)); *B. subtilis* (credit: taken from [218] © Zweers *et al.* licensee BioMed Central Ltd. 2008 (CC BY 2.0)); *E. coli* (credit: Eric Erbe, digital colorization by Christopher Pooley, both of USDA, ARS, EMU); *L. plantarum* (credit: taken from [219] and reproduced with permission from American Society for Microbiology); *M. xanthus* (credit: Jürgen Berger, Supriya Kadam, Gregory Velicer, Max Planck Institute for Developmental Biology, Tübingen, Germany); *P. aeruginosa* (credit: Janice Haney Carr, PD-USGov-HHS-CDC); *S. coelicolor* (credit: taken from [220] CC BY 4.0); *S. aureus* (credit: Janice Haney Carr, Centers for Disease Control and Prevention); *S. pneumococcus* (credit: Richard Facklam, CDC-PHIL); *S. Typhimurium* (credit: Volker Brinkmann, Max Planck Institute for Infection Biology, Berlin, Germany); *V. cholerae* (credit: Ronald Taylor, Tom Kirn, Louisa Howard, Dartmouth Electron Microscope Facility).

the need to translocate into the cell makes CDI a narrow-spectrum weapon that targets members of the same species [80]. A growing number of such contact-dependent weapon systems are being discovered, and strikingly, all appear to have independent evolutionary origins. This suggests that these complex weapons have convergently evolved multiple times across the bacterial phylogeny, testament to the importance of warfare for bacterial evolution. More recently discovered systems include the functionally similar Cdz (Contact-Dependent inhibition by glycine Zipper proteins) system [81], the type VII secretion system [82,83], and the type IV secretion system, a long-studied secretion system that was only recently found to mediate interbacterial competition [84].

Our final example of toxin delivery is the type VI secretion system, whose dry name belies its status as one of the most fascinating structures made by bacteria. Initially implicated in infections [85], the type VI secretion system was later shown to be a powerful mediator of interbacterial competition [64,86,87], and is carried by many Gram-negative bacteria [88]. Best envisaged as a poisoned molecular speargun, the type VI secretion system solves the problem of toxin release and delivery in one step by firing a toxin-carrying needle into a target cell, via a spring-loaded contractile sheath [89–91]. Cells that use the system achieve immunity to the diverse toxic 'effector' proteins carried on the needle through the expression of cognate immunity proteins, which also ensures that hitting a clonemate does not cause harm. Gram-positive species are often resistant to this type of weapon, possibly due to their thick cell walls. Nevertheless, the type VI secretion system is a versatile weapon that can kill diverse species, and there is growing evidence of its importance for bacterial competition, particularly in the mammalian gut microbiota [12,54,56,92,93].

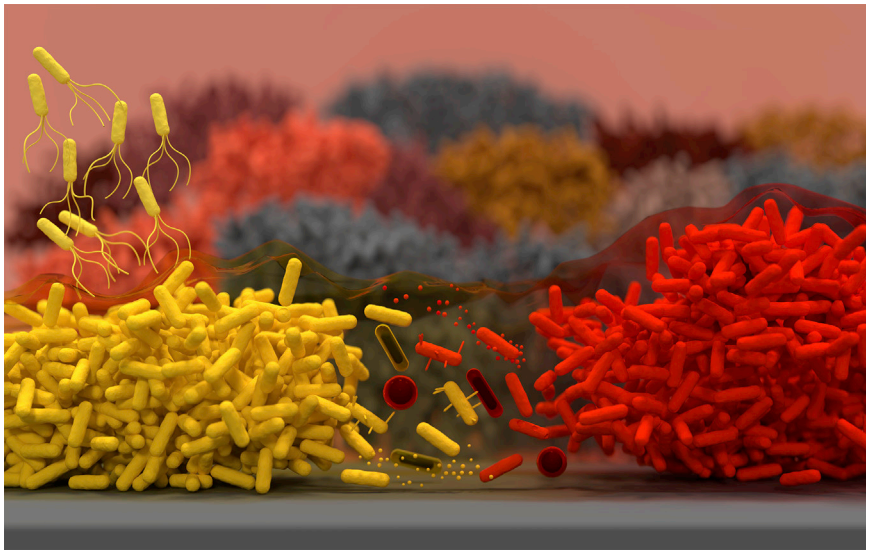

Figure 4. Illustration of warfare in a multispecies bacterial biofilm.
Bacteria commonly live in dense bacterial communities, called biofilms, where cells live encased in a self-produced, extracellular polymeric matrix, often comprised of carbohydrates, proteins and DNA. These communities can be extremely diverse, with many species and strains, but often develop distinct patches of tightly packed cells of a single genotype. Competitive interactions occur at the interface between genotypes (in the case of contact-dependent and -independent mechanisms), as well as throughout the biofilm whenever diffusible compounds are involved. Weapon use on both sides results in dead cells at the interface, shown here with a black interior and/or a rounded shape (caused by toxins that compromise the cell wall). Although dispersal from the edge of a biofilm is possible (yellow cells, top left), movement is generally restricted due to the cells being surrounded by the extracellular matrix. Artwork by Enrico Khatchapuridze.

Alternative Functions of Toxins and Delivery Systems: Metabolism, Virulence and Signalling

Not all bacterial toxins or delivery systems are antimicrobial weapons. For example, many bacteria express several toxin–antitoxin protein pairs in their cells, where the toxins degrade more slowly than the antitoxin. As a result, inactivation of expression leads to self-intoxication, which appears to be a crude but effective way to shut down metabolism and generate dormant, toxin-resistant cells (called persisters) [94]. Other toxins leave the cell but are used on eukaryotic targets during infections [95], or to overcome a single-celled predator [96]. Delivery systems, including some versions of the type VI systems, are also used to target eukaryotic cells [91,97–99], and some type VI systems also appear to be involved in metal ion uptake [100,101].

Caution must be applied, therefore, when assigning function to any bacterial toxin or delivery system. Even mechanisms that do appear to serve as weapons can have additional functions. For example, many weapons also deliver toxins to clonemates as well as competing strains. In some cases, this allows the toxin-receiving cells to detect their toxin-producing clonemates and is used to increase investment into attacks [34] or group formation (specifically biofilms; Figure 4) [102]. An extreme example of functional diversity is the redox-active molecule pyocyanin, made by *P. aeruginosa* (Figure 3). There is experimental evidence that pyocyanin can serve in bacterial warfare [43], cell–cell signalling among clonemates [103], pathogenesis [104], and metabolism (as an alternative electron acceptor for respiration) [105]. Despite each of these effects having its own literature, it remains unclear which of these are true evolutionary functions [106] in the sense that they are important for the fitness of *P. aeruginosa* in nature.

Mechanical Warfare

The weapons of humans and other animals often function via physical damage. In bacteria, the clearest examples of such weapons evolved from the viruses (phage) that infect bacteria. For example, R-type pyocins produced by *P. aeruginosa* resemble phage tails that lack the capsid shell on top that holds the viral DNA [36]. Known as tailocins, the result is a macromolecular machine that binds preferentially to sugar residues on the outer membrane of non-clonemates and physically punches a hole, which leads to massive membrane depolarization and death [107]. Like some protein toxins, the releasing cell must lyse to release R pyocins, making this again a particularly costly mode of attack. The use of mechanical weapons appears less common than chemical weapons and is only known from relatively few species [108]. However, they have a wide phylogenetic distribution and, compared to chemical weapons, they have not been looked for as intensively during the antibiotic era.

Biological Warfare

Many bacteria carry dormant viruses encoded in their genomes. Under stressful conditions, the dormant form (called the prophage) becomes activated to produce large numbers of virulent progeny, which typically leave a cell by lysis [109]. The viruses that can make a prophage are known as temperate phages and have commonly been viewed as pathogens owing to the catastrophic way they often leave a cell, and the potential for subsequent virulent replication [110]. Indeed, once activated and released from a cell, they have the potential to infect and kill vast numbers of bacteria before potentially integrating into new host genomes. However, the characterisation of temperate phages as pathogens of bacteria is questionable given a key feature of their biology. Bacteria that carry a prophage are often immune to infection by copies of the same virus through mechanisms including cell surface modifications that block viral attachment [111]. When a prophage excises itself from the genome and leaves a host cell, therefore, it will typically not harm the clonemates of that cell, as they will carry the same prophage. However, it does have the potential to infect and kill competing strains. In this way, temperate phages may function as powerful biological weapons for the strains that carry them [25,40,112,113].

The cost of carrying a prophage is that a bacterial strain will lose some cells during phage release, but the benefit is that surviving cells can experience greatly reduced competition. These

benefits may be short lived if the phages integrate into the genomes of competitors and immunise them [114]. However, if a cell carries multiple different prophages in its genome, this can reduce the probability of any given target being completely immune [115]. Are temperate phages biological weapons in the strict sense that they have evolved to enable bacteria to kill competitors, or are they simply agents of their own replication that happen to sometimes benefit bacteria [110]? This is a challenging question, and the answer rests on whether bacteria have evolved to allow, or coordinate, phage release. The potential for strong fitness benefits suggests that bacteria may indeed have evolved to enable the use of temperate phages against competitors. Broadly consistent with this, phage release is sometimes co-regulated with the production of the protein toxins used in warfare [116]. Whatever the case, prophage carriage can be a key determinant of bacterial competition, whose importance may prove to rival chemical warfare.

Other Ways to Interfere: Stickiness, Slime, Shape, Speed, and Signals

There are a wide range of other phenotypes in bacteria that allow them to interfere with each other's growth and survival without necessarily causing damage (reviewed in [2,5,117,118]). These mechanisms are more weapon-like than weapons in a strict sense. They include several physical mechanisms that allow bacteria to position and push themselves into nutrient-rich locations, including adhesion [119], the secretion of slimy polymers (extracellular polymeric substances) that allow cells to spread out and smother competitors [120,121], and even cell shape [122]. Movement is another way to gain the best position in a community [123], and there is even the potential for 'information warfare', whereby one species interrupts cell–cell signalling (**quorum sensing**) in another by consuming their signalling molecules [5,124].

The Evolution of Bacterial Warfare

Why Evolve a Weapon?

The incredible abundance and diversity of weapons in bacterial communities raises many questions for evolution and ecology. Most fundamentally, what drives the evolution of **weapons**? Close to forty years ago, two seminal papers showed that the evolutionary benefits to *E. coli* of making colicin toxins were greatest when a producer strain is locally abundant [21,125]. These presaged later studies suggesting that both local frequency and density favour toxin use, because each increases the likelihood that toxins build up enough to be effective [25,26,33,59,126–128]. Gardner *et al.* subsequently highlighted that costly toxin production was a particularly compelling example of 'spiteful' behaviour in the vernacular of sociobiology, as it is costly to the personal fitness of both the actor (toxin producer) and recipient (victim) [22]. They also made the point that, in a sense, toxin production is actually most beneficial at an *intermediate* frequency of producers — too low, and not enough toxin builds up, too high, and there is no one to kill — which was supported by subsequent experiments and agent-based modelling [20,23,24].

Toxin production may also be most favoured at an intermediate level of nutrients. Having some nutrients is important for the evolution of warfare so that a cell can afford to invest in toxin production [129,130]. However, an abundance of nutrients can also favour investing in rapid growth rather than interference, to use up the nutrients first [16]. It may be then that limited — but not too limited — nutrient conditions favour the greatest investment into toxins. Another important correlate of cell density is spatiogenetic structure, that is, the arrangement of different genotypes in space [2]. More work here is needed, but low structure — mixing of different genotypes — can make weapons more favourable as it allows attacking genotypes better access to victims, something that is particularly true for short range weapons like the type VI secretion systems [32]. That said, low structure can also correlate with a low density of any given strain [131], which — as just discussed — disfavours the use of diffusing toxins.

In summary, density, frequency, nutrients, and spatial structure are all predicted to influence the evolution of weapons. However, the effects can be complex and are likely to affect various weapon types differently. For example, whereas diffusing toxins function poorly at low cell density, releasing phages can help a rare strain to invade a community [25]. There is much then to still understand.

Tactics and Strategy: When Should Weapons Be Used?

Once a weapon has evolved, when should a cell actually use it? Decades of work on antibiotics and other toxins made by bacteria have shown that production is often tightly regulated [16,59,61,132]. However, little is known about how these regulatory networks map to the actual behaviours (or **tactics**) and **strategies** used during bacterial contests [16,34]. By contrast, there is a large body of literature on how animals behave during contests [133]. Though little discussed in microbiology [34,35], this literature is useful for thinking about how bacteria use their weapons and highlights both similarities and differences to animals.

Models of animal contests typically consider two individuals and a disputed resource to ask which decisions — such as starting a fight, staying in a fight, and retreating — are favoured by natural selection (for a detailed review see [19]). Rooted in evolutionary **game theory**, these models couch decisions in the context of a wide range of possible strategies, which might be as simple as 'always fight' or 'never fight' but also include more complex behaviours that depend on what the competitor is doing, such as 'retaliate if attacked' (known as a 'tit-for-tat' behaviour). The most discussed case is the 'hawk–dove' model, which asks whether an always-fight (hawk) or never-fight (dove) **strategy** will evolve. This makes the important prediction that fighting is not universally beneficial. Once hawks are common in the population, they will meet other hawks, leading to costly contests that can favour the persistence of doves in the population [19]. This, and later models, went on to identify several factors important in the decision to fight, including the cost of fighting, fighting ability ('resource holding potential'), and the value of a disputed resource [17,19,134,135]. Such factors seem likely to be important for bacteria as well. For example, recent work suggests that *E. coli* strains will only benefit from mounting a strong attack when they have superior fighting ability because, if not, they risk provoking a fierce counterattack that leads to a high cost of fighting [34,35].

The Importance of Information: Competition Sensing, Cues, and Signals

A major theme in the study of animal contests is the importance of information: what exactly can a focal individual glean about

the competitor and the resource, and when is this information available? For example, in animal **combat**, if an assessment can only be made by challenging a competitor, the likelihood of fighting will necessarily increase [19]. In bacteria, information is similarly critical, and the expectation is that a strain will evolve to regulate attacks based upon information that allows it to use weapons prudently but effectively. As in animals [19], we expect bacteria will tend to fight more over high-value resources than low-value ones. However, bacteria often consume resources during a contest, and therefore they may respond to resource levels in different ways to animals. For example, low nutrients can indicate high cell density and a good time to invest in weapons, and data show that nutrient stress promotes toxin production in diverse bacterial species [16]. Nutrient type can also be important. The production of some antibiotics is increased more by the limitation of a cell's preferred carbon source than by depletion of other carbon sources [136], perhaps because this indicates the most threatening form of nutrient competition for bacteria [16].

Another important source of information for a bacterium is cell damage, which may indicate an incoming attack, and there are many links between indicators of cell damage and toxin production in bacteria [16]. These observations led to the idea of **competition sensing**, which argues that bacteria commonly use their stress responses — those that detect nutrient stress and cell damage — as a means to detect and respond to competitors, both defensively and aggressively [16,132,137]. A key corollary of competition sensing is that bacteria, like animals, have evolved the ability to retaliate to incoming attacks. Further evidence of this ability comes from studies on the type VI secretion systems. Whereas many species appear to fire this weapon randomly, *P. aeruginosa* has a type VI system that is fired in response to incoming type VI system attacks, an example of an aggressive tit-for-tat behaviour in bacteria [138].

Both animals and bacteria, therefore, respond to cues from competitors when deciding whether to fight. In evolutionary biology, something is considered a **cue** when information is inadvertently provided by one individual to another, for example one strain consuming nutrients and the other detecting it [139]. This contrasts with a **signal** that evolved to allow one individual to actively communicate with another [139]. This distinction is important as true signals are a critical factor in many animal contests where contestants communicate fighting ability, social status, or intent [133,140,141]. Male red deer, for example, roar to signal their size and strength [142], and rubyspot damselflies display their bright red wing spots to communicate fighting ability [143]. There is a large literature on such signals, suggesting that they evolve to allow individuals to avoid costly fights when the outcome can be easily predicted [133]. It is unclear if bacteria have equivalents of the signals seen in animal contests [144]. Signalling one's quality requires information processing in the receiver that can both decipher the signal and also respond appropriately, and the evolution of such communication is clearly more constrained by bacterial regulatory networks than by animal brains. In addition, these signals are expected to replace weapon use, and lead to mostly peaceful outcomes, which contrasts with many studies suggesting that bacterial weapons are both extensively used and important for competitive outcomes [7,8,10–14,34,40,48–56,72–74,82,84,86,145–147].

Signalling between competitors, therefore, may prove uncommon during bacterial warfare. However, signalling between *cooperators* does occur in the form of **quorum sensing** [139,148–150], and there are many similar responses that can serve to detect or infer a quorum of clonemates [151], such as changes in pH [16]. Such communication among clonemates is commonly used to upregulate toxins, presumably because it indicates high-density conditions where toxin release is effective [5,16]. Bacteria also eavesdrop on competitors' signals: the soil bacterium *Chromobacterium violaceum* detects quorum-sensing molecules of *Burkholderia thailandensis* and upregulates production of antimicrobials [152].

Some weapons double as a means to communicate. Virulent strains of the gut bacterium *Enterococcus faecalis* release a two-subunit toxin (cytolysin) that dissociates upon binding to a range of target cells. One subunit remains and functions as a toxin, and the other is released to function as an autoinducer that activates further toxin production [153]. Cleverly, this signal ensures that toxin is only produced when sufficient numbers of both attackers and targets are present. Interbacterial communication also appears to occur via the contact-dependent growth inhibition system (Figure 1), which promotes group (biofilm) formation when it hits clonemates rather than a competitor [102]. These, and other examples [34], suggest that toxins can serve as signals and cues between clonemates, as well as weapons. What is much less clear is whether antibiotics function as signals between species to allow them to coordinate community functions, as has been suggested, motivated by the observation that bacteria show widespread regulatory responses to sub-inhibitory antibiotic concentrations [154–157]. The prevalence of competition in natural environments [1,158] suggests that these responses to antibiotics are better explained by cells preparing themselves for incoming attacks, and that the antibiotic is serving as an inadvertent cue rather than a signal between species [16,159,160].

In summary, bacteria have the capacity to integrate a wide range of information including nutrient level, nutrient type, cell damage and information on quorum in deciding when to attack. However, bacteria vary in which inputs are used — even within a species [34] — and an interesting challenge is to understand why different bacteria value different information sources differently [16].

Sociality and Sessility: When Contests Become Warfare

The way that bacteria and animals use information during contests, therefore, may differ greatly. In particular, animals are known to often resolve contests using signalling rather than fighting, something with no clear equivalent in bacteria. A second set of differences arises from the fact that many animal fights involve only two, or a few, individuals (Figure 5) [19]. By contrast, bacterial contests often occur in dense bacterial communities, known as biofilms, containing millions of individuals from many different species [161–163]. Moreover, because bacteria can undergo rapid asexual reproduction, a given cell may be surrounded by clonemates in addition to any competitors (Figure 4) [2].

Having clonemates around opens up the possibility of combat strategies that make use of **collective behaviours** involving

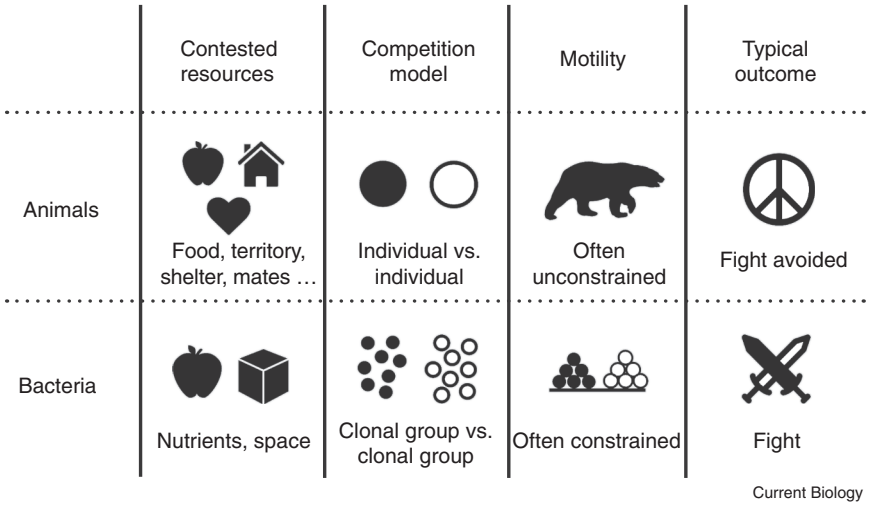

Figure 5. Key differences between the classical game theory of animal contests and bacterial warfare.
Contested resources: Animals typically compete over access to limiting resources such as food, territory, shelter and mates. Bacteria are thought to mostly compete over nutrients and space. *Competition model*: Models of animal contests have typically focused on cases with only two — or a few — individuals, whereas bacterial contests often occur in dense communities comprised of millions of individuals. Moreover, because bacteria can undergo rapid asexual reproduction, a given cell may be surrounded by clonemates in addition to any competitors. *Motility*: The ability to leave a fight is an important option in many animal contests, such as male polar bears fighting over a mate, but bacteria within large communities may become effectively sessile, as their dispersal is highly constrained. *Typical outcome*: Animals commonly evolve behaviours to avoid costly fights, including signals that allow contestants to predict the outcome of fights without actually engaging. This contrasts with what we know about bacterial contests, in which encounters are often intense and lethal.

multiple group members [164], including the use of quorum sensing to coordinate attacks, as just discussed. Cells may also warn others of incoming attacks. *E. coli* cells at the edge of a colony will sense certain incoming toxins and produce their own toxin in response. This production can be detected by their clonemates around and behind them who make their own toxin, triggering yet more toxin production to generate a coordinated and powerful counterattack (Figure 6) [34]. Although not captured by the dyadic models of animal contests that focus on just two individuals [19], such collective behaviour has a striking analogy in group-living animals, where incoming threats often drive alarm signalling. For example, all the major groups of eusocial insects — including bees, ants, wasps, and termites — have independently evolved the use of alarm pheromones that coordinate counterattacks [165].

There are further similarities between bacteria and social animals (Figure 6). A key feature of insect societies is the **division of labour**, in which different individuals perform distinct tasks. This includes the division between reproductive individuals, such as queens, and the workers who often launch vicious attacks on competitors at considerable personal cost [166]. Species like the army-ant *Eciton burchellii* show further specialisation with the production of soldier castes with larger mandibles and bigger bodies than regular workers [167]. Specialisation is also seen in bacterial warfare; antibiotic production in the soil bacterium *Streptomyces coelicolor* (Figure 3) is often performed by a subset of cells (Figure 6) [168]. Moreover, as discussed above, the insects' tendency for self-sacrifice is also seen in bacteria, with several species using cell lysis to release toxins [34,61,169]. The evolution of such traits — those that are costly to an actor's lifetime personal fitness — is the focus of a large literature on social evolution (or sociobiology [170,171]). This literature has shown that behaviours like suicidal attacks only make sense in a social group, where surviving clonemates or family members can benefit to pass on copies of the attacker's genes [22,170].

A final feature of social organisms that may contribute to increased aggression is a decreased ability to leave contests. Although dispersal from the edge of a biofilm is possible (Figure 4) [172], bacteria within large communities can become effectively sessile; so too can social insects that construct large, immovable nests. This constraint on dispersal, as well as the challenges of finding another high value site, may favour aggression to hold a territory and keep out intruders. Consistent with this, social insects are commonly extremely aggressive near their nest [165], and there is evidence for pre-emptive attacks in bacteria from the seemingly constitutive production of antimicrobials (Figure 6) [61,173,174] and contact-dependent toxins [175]. A link between sessile lifestyle and aggression is also known from sessile marine invertebrates, such as sponges, which constitutively produce toxins to kill neighbouring species (Figure 6) [176].

The aggression of bacteria, therefore, may have been promoted by their social and semi-sessile lifestyle, and perhaps also a limited ability to resolve conflicts via signalling (Figure 5). However, much is still unknown, and other factors need consideration. Central among these is the potential for complex evolutionary and ecological dynamics associated with weapons evolution.

Evolutionary and Ecological Dynamics

The diversity, abundance and common use of weapons in bacterial communities brings with it the potential for many knock-on effects. These effects are expected to be particularly important within a species as many bacterial weapons target conspecifics [59] or at least strains in the same physical or metabolic niche [177–179]. This targeting makes sense as these strains are likely to compete strongly for resources [180]. The evolution of a weapon, therefore, has the potential to set off coevolutionary dynamics between species, but the strongest effects may occur within species [181].

Arms Race Evolution

A target strain may evolve resistance to a weapon, in turn generating natural selection on the aggressor to evolve their weapons. Such **coevolution** can then lead to escalation, as in the classic arms race, where each party invests in a greater number of

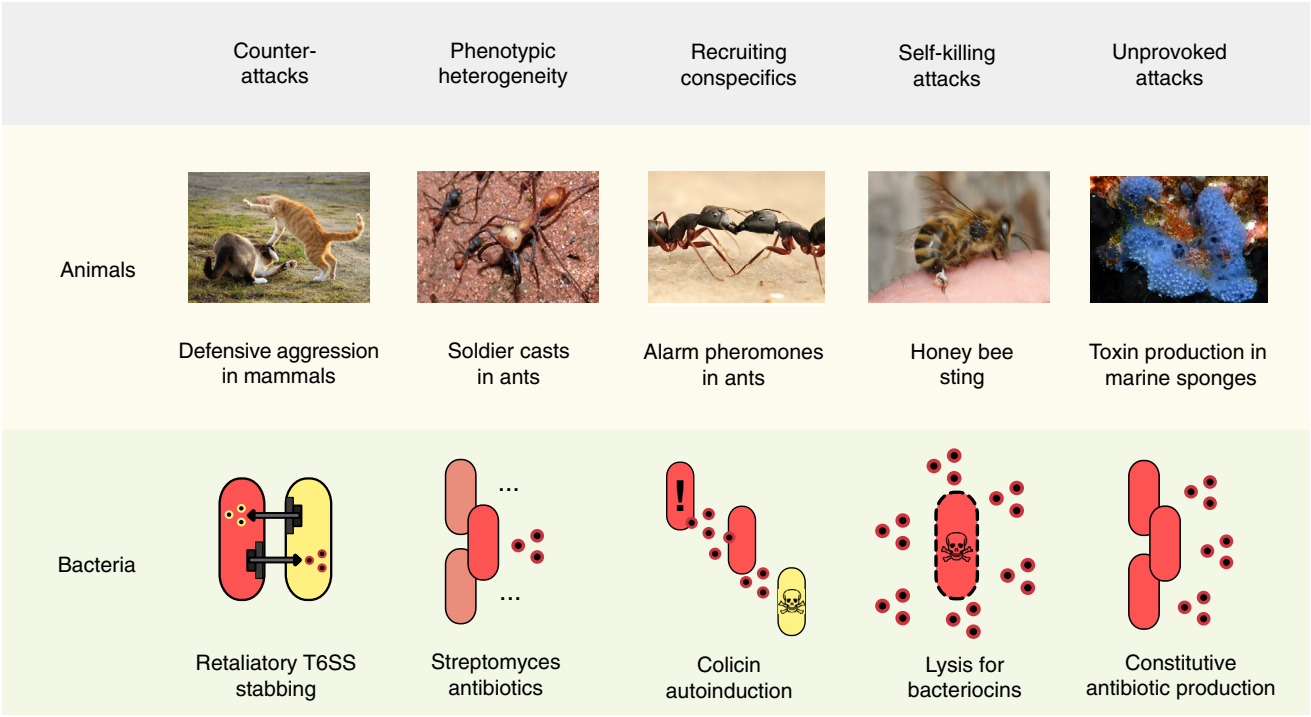

Figure 6. Combat behaviours seen in both bacteria and animals.
Counterattacks: The human pathogen *P. aeruginosa* fires its type VI secretion system into a neighbouring cell in response to an incoming attack, an example of a counterattack behaviour in bacteria [138]. Defensive aggression (retaliation in response to an attack) is also found in animals [221]. *Phenotypic heterogeneity*: The soil bacterium *S. coelicolor* uses a bistable gene switch to ensure that only a fraction of the population engages in the production of a certain antibiotic at low cell densities [168]. The army ant *Eciton burchellii* exhibits extreme physical polymorphism, where specialized soldier ants develop larger mandibles and bigger bodies than regular workers [167]. *Recruiting conspecifics*: *E. coli* cells sense incoming toxins and produce their own toxin in response. This production can be detected by their clonemates who then make their own toxin, triggering a coordinated counterattack [34]. Similarly, eusocial insects commonly use alarm pheromones to recruit conspecifics to the site of conflict [165]. *Self-killing attacks*: Many bacterial toxins — for example, colicins in *E. coli* — require the producing cell to lyse in order to be released [61]. Similarly, honeybee workers use their sting to protect the hive at a considerable cost, often leading to the worker's death [166]. *Unprovoked attacks*: Some bacteria constitutively produce antimicrobial compounds, even when growing alone and unchallenged by a competitor [174]. The marine sponge *Dysidea* sp. also appears to constitutively produce toxins to kill neighbouring species [176]. Image sources: domestic cats (credit: rihaij/Pixabay); soldier ant (© Alex Wild, used by permission); two ants communicating (credit: Rakeshkdogra/Wikimedia Commons (CC BY-SA 3.0)); honeybee (credit: Waugsberg/Wikimedia Commons (CC BY-SA 3.0)); *Dysidea* sponge (credit: Flower Garden Banks National Marine Sanctuary).

weapons and/or defences, resulting in reciprocal directional natural selection [182]. This process may have contributed to the general aggression exhibited by many bacteria, both in terms of how often, and how many, weapons are used. Identifying escalation and the evolutionary forces that drove it is challenging after the fact. However, when one strain of bacteria — specifically soil bacteria of the genus *Streptomyces* — inhibits another strain, it is more likely than average to be inhibited in return, possibly indicating the escalation of arms between competitors [183]. Particularly compelling is the case of *Streptomyces clavuligerus,* which makes multiple beta-lactam (penicillin-type) antibiotics, including cephamycin C. In many other species, resistance to these antibiotics is conferred by beta-lactamase enzymes that digest the antibiotics. However, *S. clavuligerus* has evolved to produce an additional compound, clavulanic acid, which inhibits the beta-lactamases of potential competitors [184]. So powerful is the cocktail of beta-lactam antibiotic and beta-lactamase inhibitor, it has long been used as a combination drug therapy in the clinic [185].

In addition to such specific mechanisms, the evolution of weapons appears to have driven the evolution of general defences in many bacterial species. Key candidates include the use of efflux pumps to remove toxins [67], the formation of protective biofilms (Figure 4) in response to cell damage [180], and the production of a small number of non-dividing persister cells that can survive extreme toxin concentrations [94,186].

Cyclical Coevolution, Frequency Dependence, and the Evolution of Diversity

Evolution does not always escalate [181]. The evolution of resistance by one strain can simply favour reduced use, or loss, of a weapon by an attacking strain [187]. The loss of the weapon, in turn, can favour the loss of resistance, which can itself be costly. This may lead to what is known as a 'rock-paper-scissors' dynamic that cycles through strategies of toxin production, resistance and susceptibility — a scenario that has been shown experimentally in *E. coli* [31,188]. Further diversity and complexity may be created if the resistance mechanism actively degrades the antibiotic so that one strain or species may protect others in the community [29].

Once resistance for one toxin is common, natural selection is also expected to favour strains that produce a different, rarer toxin for which resistance has not yet evolved [59]. This can

lead to cyclical evolutionary dynamics in which a large diversity of toxins is maintained in a population [27]. Consistent with rapid evolutionary turnover in weapons and defences, the strength of antagonism between bacterial strains is often poorly correlated with their phylogeny [177,178,189]. Moreover, while a given strain will often produce inhibitory factors, it may only kill a small subset of potential target strains [177,183], perhaps because it may be cheaper to carry genes for resistance than for weaponry [178]. This raises the intriguing possibility that coevolutionary dynamics may lead to a situation where, at any one time, bacteria are investing heavily in weapons that are largely ineffective.

Central to many models of weapon turnover is what is known as negative frequency-dependent selection, whereby rare strategies tend to have an advantage. Negative frequency dependence is a general process that generates genetic diversity [181], and is a major theme in the evolutionary game theory of animal combat, where a diversity of strategies is commonly predicted (mixed evolutionarily stable strategies) [17,19,34]. Whereas the benefits of having a rare toxin can generate negative frequency dependence, at smaller spatial scales there is also the potential for positive frequency-dependent selection. This is because, as discussed, a locally abundant strain can generate high toxin concentrations to kill competitors [21,125]. This effect can remove diversity locally but, when combined with stochasticity in which genotype colonises each position, the result can be a patchwork quilt where each genotype dominates locally but not further afield. Such spatial ecology appears to mirror coral reefs, where clonal groups of coral polyps exist in close proximity [190–192] and stochastic immigration, competition and chemical warfare are important [193,194].

Horizontal Gene Transfer and Multi-Level Selection In Bacterial Warfare

Unlike animals, bacteria are often able to pick up and express small pieces of DNA via horizontal gene transfer. This, and the modular organisation of bacterial genomes, means that bacteria have the potential to rapidly gain and lose a wide diversity of weapons. Weapon acquisition can even occur during contests, as some species co-regulate DNA uptake with the use of chemical weapons [195,196]. A similar link is seen in certain temperate phages that, upon infecting a new bacterial strain, generate some phage particles containing DNA of the bacterial victim instead of their own phage DNA. These particles are then picked up by the original bacterial host in large numbers allowing the killer to widely sample the DNA of the victim [197]. In these examples, weapons can then be used to steal the weapons and defences of competitors, as well as to kill [198].

Horizontal gene transfer has the potential to strongly shape bacterial communities by allowing diverse strains to acquire the same phenotypes [199]. Such horizontal acquisition of weapons and defences may explain the surprising result from marine bacteria that strains from a given community are less likely to kill each other than those from other communities [200]. Indeed, many bacterial weapons are associated with mobile genetic elements [59], which means that the ecology and evolution of weapons can be partially decoupled from the bacteria that carry them. For example, a plasmid for a given toxin may occur in a niche that is overlapping with, but distinct from, all of its bacterial hosts [199]. Evolutionarily, there can be conflicts of interests between the mobile elements carrying the weapons and their bacterial hosts, something that is most clear when a prophage of one bacterial strain integrates into a competing strain and immunizes it from further attack [114]. The potential for multi-level selection to shape the evolution of mobile genetic elements has long been recognized [201], but its impact on bacterial warfare remains little explored.

Conclusions and Applications

Microbes affect many aspects of our lives; they shape ecosystems, agriculture, industrial processes, health, and disease. There is particular interest in the plant- and animal-associated communities that are central to the health and welfare of their host [202–205]. The study of bacterial warfare can help with understanding and manipulating these vital communities [206].

Most simply, possession of a weapon can predict whether a particular pathogen will invade or a symbiont persist [7–14,48–56,207,208]. Weapon use also has the potential for complex effects that ripple through communities, as each species may affect multiple others via nutrient competition and release, in addition to warfare [2–6,118,158]. Host-associated communities also enable audacious forms of bacterial warfare that work by provoking the host to attack the whole community [209]. For example, the gut pathogen *Salmonella enterica* serovar Typhimurium uses a sub-population of cells to invade the host gut epithelium and provoke a massive immune response. This devastates the bacterial populations in the gut, but the remaining *S.* Typhimurium use a chemical byproduct of the immune response to respire and rapidly proliferate [210]. A challenge looking forward is to understand the causes and consequences of warfare within such complex ecological systems, both for individual species and for system-level properties like community stability [29,211].

We also need ways to manipulate microbial communities. Broad-spectrum antibiotics are commonly used to suppress pathogens, but they also harm many other species in the microbiota. Considering the full diversity of bacterial weapons reveals many narrow-spectrum alternatives, such as the protein bacteriocins [212]. Further specificity may be achieved by identifying probiotic species that release toxins right next to a pathogen being targeted [51,206]. Probiotics also have the potential to evolve, raising the possibility that resistance evolution will be less of an issue than for antibiotics. The evolution of resistance will nevertheless be a problem, both for drugs and for probiotic strategies. The study of bacterial behaviour during contests can help, as some species reveal new toxins, and mechanisms of drug resistance, when in competition with other species [16,132]. Finally, there is the intriguing potential to treat disease with small amounts of drugs that provoke bacteria to fight, and thereby eliminate one another [35].

For close to a century, microbiologists have studied mechanisms of bacterial warfare [213]. This has revealed an astonishing abundance and diversity of weapons, many of which are used despite considerable costs of deployment. Understanding the causes and consequences of bacterial aggression is an open challenge for evolutionary biology and ecology. And as we

seek to remove problem strains, or manipulate microbial communities, we would do well to remember that bacteria have spent the last three billion years doing just this.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure, one table and supplemental references and can be found with this article online at https://doi.org/10.1016/j.cub.2019.04.024.

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