Bacterial species rarely work together

Bacteria often compete, offering alternatives to antibiotic therapy

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Bacteria commonly live in diverse communities where each species can impact the growth and survival of other species(*1*, *2*). These species interactions are central to the ecology of bacteria and their many impacts in health, agriculture, and industry. To understand and manipulate bacterial communities, therefore, it is critical that we understand how species interact. However, there is an emerging controversy on the importance of positive interactions, such as cooperation, between bacterial species(*3*–*8*). Here we discuss the data and show that once one applies the standard ecological measures, a clear picture emerges. Negative interactions prevail and cooperation between species is typically rare. These findings suggest that bacteria - and those that wish to engineer bacterial communities for human benefit – must contend with competition and conflict.

Species affect each other in many ways (Figure 1). Antagonistic interactions like competition, parasitism and predation are rife with conflicts where natural selection pushes each party towards different outcomes. An owl benefits when it can eat a storm petrel (Figure 1), while the petrel benefits by not being eaten. Cooperative interactions, where both parties benefit, are characterised by natural selection on species to work together, where each achieves something that they cannot do alone. Plants disperse pollen via the flight of the bumblebee, while the bee obtains nectar via the plant's ability to fix atmospheric carbon. Species interactions are also critical for how communities behave as a system, influencing community diversity, assembly, stability and productivity. For example, cooperation (+/+) can destabilise ecological systems by causing dependencies, while exploitation (+/-) can be the most stabilising ecological interaction because it creates a negative feedback between species(*9*). It is critical then that we understand how species interact in order to understand them and the communities that they form.

Among the ecological interactions, cooperation holds a special status in the literature(*2*, *10*). At first sight, cooperation can be puzzling: why would an individual invest in another over itself? There is now a vast literature dedicated to answering this question, both in general(*10*) and for microbes in particular(*1–3*, *8*). A key finding is that cooperation readily evolves in clonal groups of microbes because they share evolutionary interests, like cells in a multicellular organism(*2*). However, between species, cooperation (mutualism) is predicted to be much less common because of natural selection for competition and exploitation when different genotypes are interacting(*1*, *2*, *10*).

One can, in principle, test this prediction using genomic data and computational methods that try to infer how different bacteria will interact(*2*). However, the gold standard is to culture bacterial species alone and together and directly assess their ecological interactions (Figure 1a). Almost a decade ago, one study did such culturing with environmental bacteria isolated from treeholes(*8*). The authors concluded that cooperation was uncommon, just as theory predicts. However, with just one study on one environment, it was not clear whether this finding would be robust to different environments and methodologies(*3*). Recent work has provided experimental data on bacterial interactions from a range of environments, including the mammalian gut, nematodes, the phyllosphere and soil. How do these new data line up with the treehole study? The majority of studies closely align with the treehole data, concluding that cooperation is rare. However, not all studies reach this conclusion. A new study of soil bacteria instead argues that positive interactions are common among culturable bacteria(*3*). Why are studies reaching such different conclusions?

To understand what is going on, we should look in more depth at the new studies. Weiss *et al*(*4*) tested a set of twelve bacterial species that colonise the mouse gut, known as the Oligo-MM12 community. Pairwise interactions between the 12 species were quantified *in vitro* in a well-defined, nutrient-rich environment. The data support the prediction that cooperation is rare, with the most common interaction type being ammensalism (negative effect in one direction, -/0), followed by neutral (0/0) and competitive (-/-) interactions. In fact, of the 66 interactions measured, none were found to be cooperative, and just a single interaction was commensal (+/0). Consistent with this finding, a second *in-vitro* study of human gut bacteria found that negative interactions dominate(*7*). There were differences in that many fewer neutral (0/0) and more exploitative (+/-) interactions were found than in the Oligo-MM12 study. These shifts in the estimates underline that different studies can yield quite different results, which may be due to differing methodologies as much as real ecological differences between communities. Nevertheless, both studies support the idea that cooperation (+/+) is rare, with cooperation absent in the Oligo-MM12 study and estimated at around 6% of interactions in the other study. This rarity is also supported by *in vivo* experiments with bacteria in nematodes and plants. In nematodes, pairwise experiments revealed that competition was by far the dominant interaction, with cooperation again the rarest at around 2% of interactions(*5*). The plant study was done differently to the others in that - rather than comparing strains alone and in pairs - the authors assessed interactions based on the removal, or addition, of single species in a diverse community. With this method, one can only assess ecological effects in one direction in a given experiment but, even so, negative interactions again predominated(*6*).

This is where things get less clear. Kehe *et al*(*3*) used droplet-based culturing to test bacterial interactions between 20 species of soil bacteria. With this method, the authors surveyed 180,000 interactions across 40 different environments, and report >40% of the cases tested contained positive interactions. This result is contrasted against the older environmental data from treehole bacteria(*8*), where Kehe *et al* note that evidence of positive interactions was found in <10% of pairs of bacteria(*3*). With such a shift in the estimates, the new data appear to change everything. However, in reality, the new study(*3*) has simply made a subtle but critical change in how one defines “positive interactions”. While the treehole study(*8*) focused exclusively on cooperation (+/+), where two species both help each other, the new statistic instead counts all cases where there is *at least one positive interaction*(*3*). This is problematic, as it groups exploitative interactions (+/-) like parasitism and predation with cooperation (+/+), which have fundamentally different evolutionary and ecological properties(*2*) (Figure 1a). Moreover, as a result of the change in definition, the authors’ suggestion that previous work(*8*) had found evidence of positive interactions in <10% cases becomes misleading. The prior 10% estimate concerned only cooperation (+/+), and it said nothing about cases with only one-way positive effects (e.g. exploitation +/-, or commensalism +/0). The importance of this distinction jumps out when one looks for the frequency of cooperation (+/+) in the new data: a mere 5% of interactions(*3*) (Figure 1b). The estimate from the new data is in fact lower.

There is understandably a fascination with cooperation and positive interactions between species, and this fascination can be leveraged in public communication of science, or business ventures. However, whichever measure of ecological interactions one uses, the Kehe *et al*(*3*) dataset suggests again that negative interactions are much more common than positive ones (Figure 1b). Such statistics do not mean, of course, that cooperation never evolves between bacterial species. There is evidence that cooperation proper (+/+) can evolve between gut species(*11*), even if it is generally uncommon in the gut. Moreover, one study found evidence for several examples of cooperation in a small bacterial community living in toxic metal working fluid(*12*). This study is important as it shows that the likelihood of bacterial species working together can greatly increase in certain environments. In this case, low species diversity and the challenges associated with growing in a harsh environment were found to be crucial. When nutrients or additional species were added, the interactions shifted to become more negative(*12*). Consistent with the importance of diversity, a study of bacteria in the *Drosophila* gut found a number of positive interactions when species were cultured in pairs, but these nearly all shifted to become negative when diversity was increased to more natural levels(*13*). Indeed, the original treehole study discussed above also found that the impacts of negative interactions increased with diversity(*8*), presumably because more species must now compete over limiting nutrients(*12*).

Looking forward, there is a need for further surveys of species and environments if we are to understand the general characteristics of bacterial communities. Nevertheless, the emerging pattern is that cooperation is relatively rare with negative interactions predominating (Figure 1b). Another important pattern in the data is the considerable variability in the strength of ecological interactions, with many being relatively weak(*3*, *4*). Like negative interactions, weak interactions are predicted by basic evolutionary models: strong competition between species is predicted to drive one species extinct or character displacement that weakens the interaction(*2*). However, in another sense, these weakly negative interactions present a conundrum. Many bacteria use powerful antibacterial toxins against one another, which can cause very strong ecological effects(*1*). How is this observation reconciled with so many weak interactions? The answer lies in the fact that antibacterial toxins often target members of the same species, which vie for the same nutrients and locations. These strongly negative interactions take place between different genotypes (strains) of the same species and are typically not captured in the ecological surveys to date. Intraspecific interactions deserve more attention and are needed for any complete picture of bacterial community ecology.

The emerging patterns on the ecology of bacterial communities have implications for those that seek to manipulate and engineer microbial communities for human benefit. Negative and weak interactions can be desirable from a community engineering standpoint as both can promote stability(*9*). Indeed, a key feature of the human gut microbiome is its relative stability, which is what allows it to typically recover from perturbations such as antibiotic treatment(*9*). Negative interactions can also drive priority effects, where late arriving species are unable to establish due to the effects of early arriving species(*6*). Competition and exploitation, therefore, can make the addition of new species challenging. This effect is well known from studies of probiotic use, where bacterial species often fail to colonise and only exist transiently in the person that ingests them(*14*). However, such colonisation resistance can also be to our benefit when incoming bacteria are harmful. With rising levels of drug resistance in pathogenic bacteria, alternatives to antibiotics are urgently needed. There is currently a great interest in finding bacterial species that both colonise the human microbiome and compete strongly with pathogens(*15*). The hope is that we can introduce these competitive species as a prophylactic, or even as a treatment to eliminates pathogens if we can master the ecology of species replacement in the microbiome. The route out of the crisis in antibiotic resistance, therefore, may rest upon both the prevalence and power of bacterial competition.

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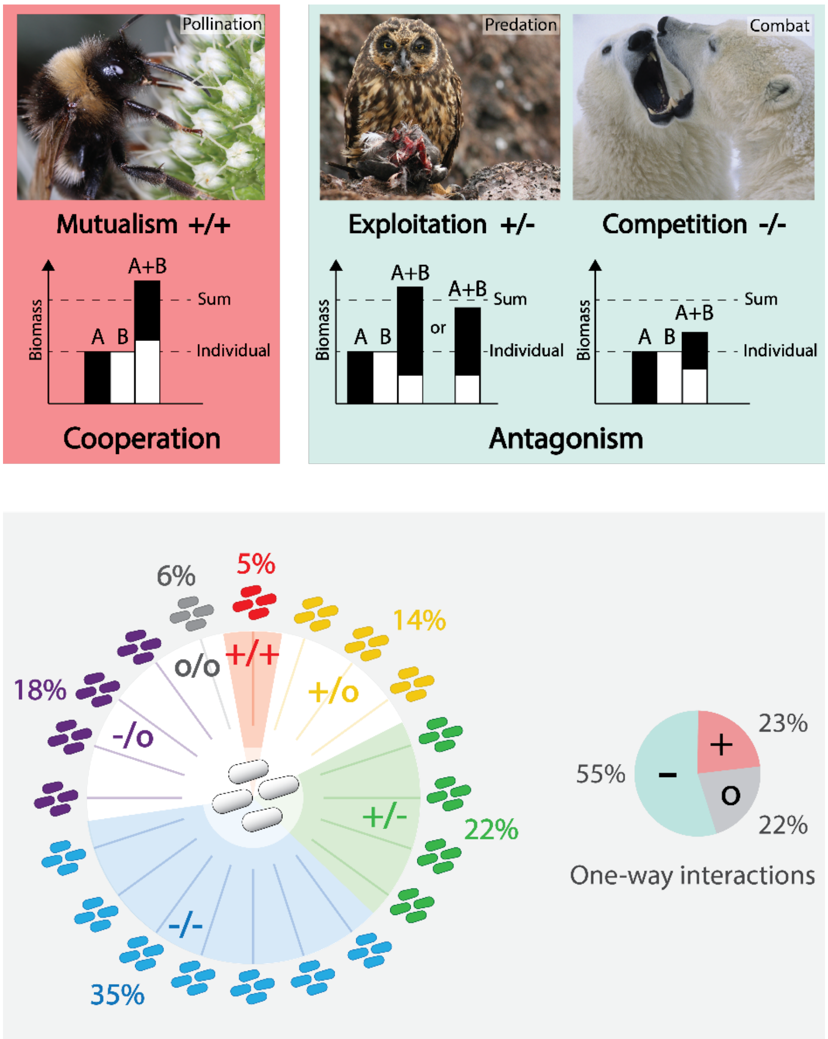

Figure 1) Competition and exploitation are common between bacterial species, while cooperation is rare. Top shows interaction types with illustrative examples, where the bar charts show hypothetical data used to diagnose each type of interaction(*2*, *8*). Bottom shows distribution of ecological interactions found in the data of ref. (*3*), both for two-way measures and one-way measures e.g. +/- interactions count equally as one positive and one negative interaction. Bee and owl photos by K. R. Foster.