The Genotypic View of Social Interactions in Microbial Communities

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Abstract

Dense and diverse microbial communities are found in many environments. Disentangling the social interactions between strains and species is central to understanding microbes and how they respond to perturbations. However, the study of social evolution in microbes tends to focus on single species. Here, we broaden this perspective and review evolutionary and ecological theory relevant to microbial interactions across all phylogenetic scales. Despite increased complexity, we reduce the theory to a simple null model that we call the genotypic view. This states that cooperation will occur when cells are surrounded by identical genotypes at the loci that drive interactions, with genetic identity coming from recent clonal growth or horizontal gene transfer (HGT). In contrast, because cooperation is only expected to evolve between different genotypes under restrictive ecological conditions, different genotypes will typically compete. Competition between two genotypes includes mutual harm but, importantly, also many interactions that are beneficial to one of the two genotypes, such as predation. The literature offers support for the genotypic view with relatively few examples of cooperation between genotypes. However, the study of microbial interactions is still at an early stage. We outline the logic and methods that help to better evaluate our perspective and move us toward rationally engineering microbial communities to our own advantage.

THE EVOLUTION OF SOCIAL INTERACTIONS

Social interaction: fitness effect of one cell on another. A subset of interactions is social adaptations that evolved, at least in part, because of their effects on others

Strain: a genetically identical set of cells (clone-mates)

The study of social interactions has traditionally been dominated by experiments and observations of macroorganisms, such as birds and bees (7). However, it is now well established that microorganisms, particularly bacteria, spend much of their time in dense, surface-associated communities that contain many strains and species (43, 87, 97) (**Figure 1**). Cells in these communities live in close proximity and display a large array of phenotypes that strongly affect the survival and reproduction of the cells around them. Understanding these interactions is as central to microbiology as it is to the study of higher organisms. Do microbes evolve to cooperate or compete?

We seek then to understand and predict the evolution of social adaptations in microbes. Social adaptations are microbial phenotypes that affect other cells and that evolved, at least in part, because of their effects on others. Examples include the secretion of enzymes to help other cells and the release of toxins to kill and

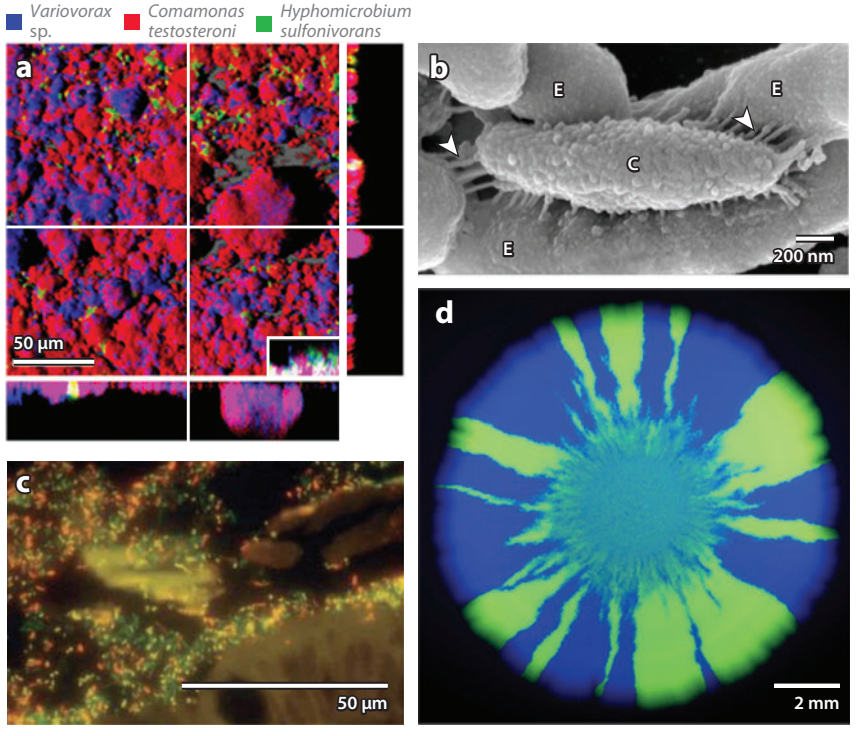

Figure 1

Examples of microbial communities. (*a*) Confocal microscopy image of a consortium biofilm with a *Variovorax* sp. (*blue*), *Comamonas testosteroni* (*red*), and *Hyphomicrobium sulfonivorans* (*green*) grown in a flow chamber. Adapted from Reference 8. (*b*) Electron microscopy image of the two-species aggregate *Chlorochromatium aggregatum*, showing photosynthetic sulfur bacteria (E) attached to a betaproteobacterial cell (C) by thin filaments (*arrows*). Adapted from Reference 150. (*c*) Multispecies microbial biofilm covering the mucosal surface of a human gut, stained using triple-color fluorescent in situ hybridization. Adapted from Reference 137. (*d*) A bacterial colony inoculated from a mixture of green-fluorescent-protein- and cyan-fluorescent-protein-labeled but otherwise identical type IV pili and flagella mutants of *Pseudomonas aeruginosa* grows to form sectors of clonal groups.

compete with neighbors. We expect that such social adaptations drive the majority of strong interactions in microbial communities and that their net effects are central to properties such as productivity, stability, and the ability to resist disturbance (135). Such properties are of considerable interest when we come to manipulate microbes to our own benefit, such as by disrupting pathogenic communities with antibiotics or by promoting efficiency in communities that produce energy or break down waste.

Our goal in the first part of the review is to outline the state of the theory of interactions among microbes. We emphasize that asexually reproducing microbes can generate patches of genetically identical cells, which inclusive fitness theory tells us have a common evolutionary interest (45) (see sidebar, Inclusive Fitness Theory and Relatedness). Within these patches, cooperative adaptations of one cell that increase the fitness of another cell are favored by natural selection, as long as this increases the total fitness of the cells of that genotype. The conditions for cooperation within a genotype, then, seem relatively common in microbes. The situation changes when a microbial group contains multiple genotypes. Natural selection is no longer expected to maximize group fitness because each genotype has its own evolutionary interests. Here, we incorporate a theory that considers the impact of ecological competition between genotypes. A focal genotype is expected to increase the fitness of another genotype only when the return benefits outweigh any costs of ecological competition between the genotypes. In contrast to within-genotype cooperation, the conditions for such cooperation between genotypes seem relatively rare.

This leads us to our key distinction—within and between genotypes—which forms the basis of our genotypic view of microbial interactions. This null model predicts cooperative adaptations between cells that are identical at the loci driving social interactions, whereas cells that differ at these loci typically exhibit competitive phenotypes. In this view, cooperation between genotypes does occur but is relatively uncommon, and the majority of positive interactions are in one direction only, as occurs when one genotype exploits and harms another. In the second part of the review, we evaluate the empirical evidence for the genotypic view. The study of microbial interactions is still at an early stage, and the final section discusses the logic and tools required to better dissect the effects that microbes have on each other.

INCLUSIVE FITNESS THEORY AND RELATEDNESS

Here, we briefly review the foundational theory and definitions of sociobiology, which are covered extensively elsewhere (e.g., 27, 45, 96, 123, 156). Inclusive fitness theory focuses on a particular social phenotype—one that has fitness effects on others—and asks whether it is favored by natural selection. This is answered by classifying social phenotypes on the basis of their direct (personal) fitness effects: selfishness (positive effect on actor, negative on recipient), mutual benefit (positive effect on both actor and recipient), altruism (negative effect on actor, positive on recipient), and spite (negative effect on both actor and recipient). Selfishness and mutual benefit are explained by benefits to the actor, such as a fighting polar bear winning a mate or a pollinator gaining nectar. Altruism and spite are more challenging but can evolve when individuals that are genetically related to the actor benefit from the action. But what does related mean?

Intuitively, relatedness can be thought of as the probability that the benefits of a social phenotype fall upon carriers of the genotype that drives the social phenotype (the probability of within-genotype benefits). In animals, this association typically comes about through family life, which is well evidenced in altruistic insect workers (27). In asexually dividing microbes, relatedness approximates to the probability that a cell affected by a social phenotype is identical at the loci generating the social phenotype. Identity at these loci can be due to cells sharing a recent common ancestor or to horizontal gene transfer (see sidebar, Horizontal Gene Transfer). The key is that a genotype that permanently harms a focal cell can only be favored by natural selection when this somehow benefits other copies of the genotype in other cells. This is exemplified by cells spitefully committing suicide to release toxins that kill other genotypes (98), which requires strong benefits to surviving cells of the suicidal genotypes.

Cooperative adaptation (cooperation): phenotype that increases the fitness of another cell and that evolved at least in part because of this effect

Competitive adaptation (competition): phenotype that reduces the fitness of another cell and that evolved at least in part because of this effect

Genotype: genetically distinct set of microbial cells. In particular, groups of cells that are identical at the loci for a social phenotype

Ecological competition: negative effect of one cell on other cells' survival and reproduction, which may or may not be the result of an evolutionary adaptation

Altruism: adaptation that improves the fitness of other cells and, in so doing, causes a reduction in personal fitness in the focal cell, e.g., reduced rate of cell division

Cheater: genotype that makes use of benefits provided by other genotypes without contributing, at a cost to the providing genotypes

Relatedness: a concept used to make social evolution predictions. Relatedness is most usefully assessed as genetic similarity, above the population average, at loci determining a social phenotype

COOPERATION OR COMPETITION? A REVIEW OF THE THEORY OF MICROBIAL INTERACTIONS

Interactions Among Cells of One Genotype

Clonal microbial cells have a special status in evolutionary theory, as, like the cells in our body, they have a common evolutionary interest (7, 45, 96, 156) (see sidebar, Inclusive Fitness Theory and Relatedness). According to inclusive fitness theory, natural selection is expected to favor whatever phenotypes maximize the overall survival and reproduction of the genotype. The specialness of a clonal group of cells is most clearly seen when it drives the evolution of so-called altruistic traits that harm a focal cell to promote reproduction of other group members (see sidebar, Inclusive Fitness Theory and Relatedness). Such extremes of cooperation mirror the self-sacrifice seen by sterile workers in social insect colonies.

A wide range of additional phenotypes that appear to be consistent with altruism occur in microbes (153) (see sidebar, Inclusive Fitness Theory and Relatedness). These include the secretion of extracellular enzymes that digest otherwise inaccessible compounds (40, 148) and of siderophores that allow cells to harvest poorly soluble iron (103, 111). More subtly, microbes also seem to regulate their growth to increase the efficiency of collective resource utilization (75, 145). Many of these cooperative phenotypes are further regulated through quorum sensing (19, 22, 56, 152), whereby cells secrete signaling molecules that allow neighboring cells to assess the population density of the clonal group (56, 97). Finally, in some species there is evidence of differentiation whereby cells perform specialized functions during biofilm development (144).

Over the past decades, it has been established that altruistic cooperation within groups containing a high frequency of clone-mates is likely to be widespread (for reviews, see References 16, 96, 153, 156). However, such cooperation is in no way guaranteed, as it relies on the benefits of cooperative traits being efficiently shared between members of a single genotype. Loss-of-function mutations at the loci driving cooperative phenotypes can generate so-called cheater genotypes that benefit from the cooperative trait without contributing (49, 142). The spread of cheater genotypes relies on a degree of genotypic mixing, allowing the cheater cells to make use of the cooperation of other cells (41, 96, 156). This leads to the key prediction that natural selection for a social trait that has a positive effect on neighboring individuals increases as a function of relatedness to those individuals, where relatedness approximates to the frequency of clone-mates (45, 96, 155, 156) [**Figure 2*a***; see sidebar, Horizontal Gene Transfer, for qualification in the case of horizontal gene transfer (HGT)]. A stable coexistence of the cooperator and cheater strains can sometimes occur because the benefit of cheating is reduced with an increasing number of cheaters (17, 71, 122).

The potential for costly cheater mutations to arise and then invade also means that we expect mechanisms that limit the loss of the cooperative phenotype (138), such as pleiotropic effects, whereby genes coding for the cooperative phenotype are linked to other essential functions (18, 29); policing, whereby cheater cells are inhibited (76, 138); or the regulation of the cooperative phenotype, such that it is only expressed when it is least costly (161).

In sum, whenever social phenotypes can be preferentially directed at the same genotype, altruistic cooperation is expected to be both common and stable. Indeed, we argue here that this mode of cooperation between bacterial cells is likely to explain the majority of cooperative microbial phenotypes. Accordingly, the frequency of clone-mates in a particular region around a focal cell may be a good first predictor of the potential for cooperation at that scale (96).

Interactions Among Cells of Different Genotypes

Inclusive fitness theory predicts that cooperation within a single genotype should be

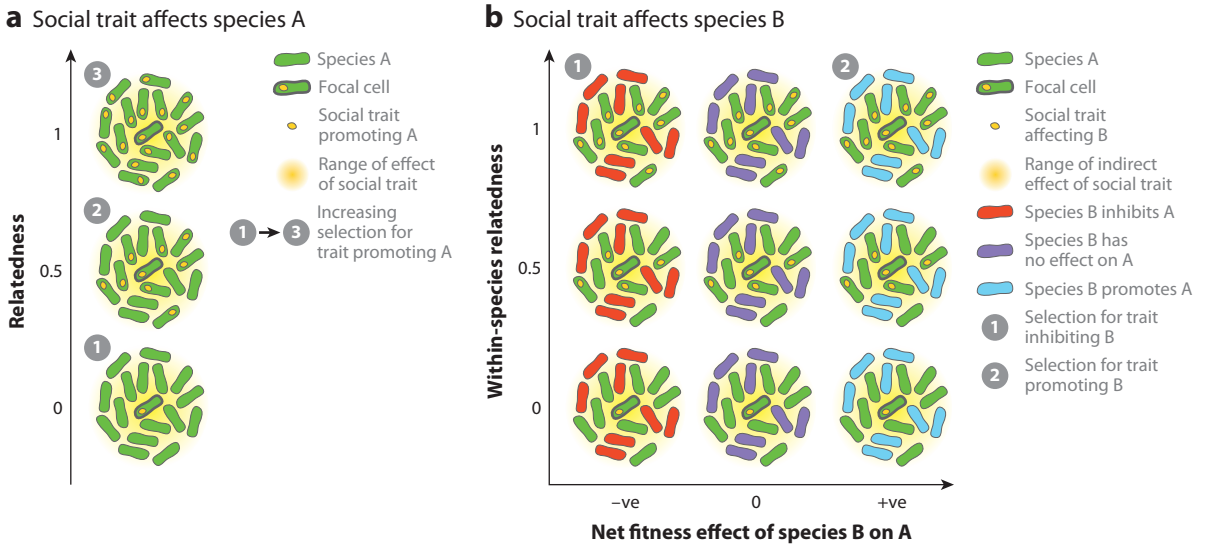

Figure 2

Selection on social traits within and between species, in which species is meant only as a proxy to distinguish phylogenetically close (within species) and phylogenetically distant (between species) strains of microbes. (*a*) Within species: Assuming that interacting cells share the same niche, the evolution of cooperation is determined purely by sharing a common genotype at the locus driving the social trait. This predicts cooperative adaptations at high relatedness (within genotypes). (*b*) Between species: Can cooperation between phylogenetically distinct cells emerge? In the simple graphic, species B may either inhibit or promote the growth of the focal species A, depending on niche overlap and secreted products. If species B is harmful (*left column*), species A is selected to increase competitiveness in return. Relatedness within species A then determines whether exploitative competition, such as less efficient resource use (low relatedness), or interference competition, such as costly toxin secretion to target species B (high relatedness) will be selected for. If species B is helpful (*right column*), reciprocal cooperation is not assured and also requires high within-species relatedness (*top right*). This double constraint—requiring both help by species B and relatedness in species A—may mean that cooperation between phylogenetically distant genotypes is relatively unlikely to emerge. Along with selection on within-species social traits, this forms the basis for the genotypic view as a null model of microbial interactions.

common (45, 96, 156) (see sidebar, Inclusive Fitness Theory and Relatedness). Moreover, in its basic form, the theory also predicts the converse: Competition should prevail between different genotypes, be these different strains with a recent common ancestor (**Figure 2*a***) or phylogenetically distant species. However, this prediction comes with the implicit assumption that interacting genotypes are competing for resources, which may not always be the case. Instead, one needs to bring in a more careful consideration of ecology. Interactions in microbial communities can be viewed from the perspective of two fields that have developed largely separately: ecological theory and social evolution theory (36). Although social evolution theory has been quite successful at predicting the evolution of social phenotypes, it puts less emphasis on the ecology in which these organisms are evolving. This is because the focus is often on interactions within one species in which the ecology can be summarized by measuring the benefits and costs of a social trait. In order to apply the theory to microbial communities with multiple interacting species, there is a need to extend social evolution theory to more explicitly include the effects of ecological competition and vice versa.

Classical ecology considers how the degree of competition between strains or species can be understood in terms of the degree of overlap in their niche (1, 33, 48, 74), with strong overlap leading to strong resource (exploitative) competition and no overlap leading to no

Horizontal gene transfer (HGT): transfer of genetic material between two cells in a manner not involving cell division

HORIZONTAL GENE TRANSFER

Our discussion focuses on how genetic identity at a focal social genotype arises through clonal propagation of a particular genotype. However, microbes can share genetic elements, which often code for secreted proteins (100), with other cells through horizontal gene transfer (HGT) (32, 102). Although the probability of HGT decreases with phylogenetic distance, it can nevertheless lead to a gene driving a social trait to move from one strain to genetically different strains. This emphasizes the need to focus on the loci that drive a social phenotype when making evolutionary predictions about that phenotype. Indeed, at that locus, our basic predictions remain unchanged: Genotypes that are the same cooperate, and genotypes that are different compete (133) (**Figure 3**).

Because the two strains differ at other loci, however, the evolutionary prognosis can be different from clonal groups. In particular, if a modifier that inactivates the social trait is more likely to spread by natural selection and/or HGT in one strain background than another, then this can undermine cooperativity. Such a modifier may also spread through both strains by HGT with similar effect (83). More complicated scenarios can also be imagined, and the effects of HGT on social interaction are an interesting area for future research.

effects on one another. We distinguish between strains and species to distinguish between genotypes that differ at the locus that drives a social trait but are otherwise largely similar across the genome (strains, with or without yellow allele in **Figure 2**) versus genotypes that differ in many traits (species, different colored cells in **Figure 2**). We recognize the difficulties with this distinction for intermediate cases (32, 102). Nevertheless, it provides a useful shorthand that maps to social evolution theory, with its focus on interactions within species, and also the branch of ecological theory that considers between-species interactions in terms of niche overlap. The sidebar Horizontal Gene Transfer shows how the logic of **Figure 2** can be generalized in a way that does not rely on these definitions.

Exploitative competition: ecological competition over resources, e.g. one genotype consumes resources and reduces the resources available to another genotype

Interference competition: ecological competition involving direct harming of one genotype by another, e.g. toxin production

Here, we take the concept of ecological competition and generalize it to include all possible net fitness effects of a second species B on a focal species A (**Figure 2*b***), which can include negative effects from niche overlap but also positive effects if species B provides some service that promotes the growth of species A in some way. We then ask the following question: When will species A evolve to harm (competitive adaptation) or help (cooperative adaptation) species B? Specifically, we focus on the fate of an allele (the yellow dot inside the cells in **Figure 2**) that causes an effect that can either harm or help species B. For simplicity, we assume that any helping or harming from the focal allele does not directly affect species A cells.

A first general prediction is that whenever the focal cell is surrounded by ecological competitors of species B (left column of **Figure 2*b***), natural selection tends to favor competitive traits that benefit the focal cell only, such as rapid and wasteful growth (exploitative competition). However, the focal cell can also display phenotypes that directly harm (interference competition) or help (cooperation) species B cells. In our scenario, the effects of these phenotypes fall back on all species A cells within the group. To understand whether direct harming or helping of species B is favored by natural selection, we must also consider relatedness within species A.

At (near) zero relatedness (bottom row of **Figure 2*b***), the focal cell is surrounded by species A cells that do not carry the allele for the social phenotype. This means that any benefits of directly helping or harming species B will also fall back on species A cells lacking the allele. Why is this important? Consider that the focal cell releases a secreted toxin that inhibits competing species B cells (bottom left of **Figure 2*b***). The other species A cells do not carry the allele or pay for costly toxin secretion, but they do receive the resource benefit from the harming of species B. The toxin-secreting cell will be outcompeted. When relatedness is low, therefore, natural selection does not favor phenotypes that directly harm (interference competition) or help (cooperation) species B cells (30).

The situation is different when all species A cells possess the allele and are genetically

identical at the locus for the social trait (top row of **Figure 2*b***). Any benefits of phenotypes that affect species B now reliably feed back on the cells carrying the allele. Accordingly, when species B is competing with species A (top left of **Figure 2*b***), natural selection tends to favor harmful traits in species A, such as antimicrobial production (although see sidebar, Helping Enemies, Harming Friends). By contrast, when relatedness is high and species B promotes growth in species A (top right of **Figure 2*b***), natural selection favors the evolution of cooperative adaptations to help species B. Intermediate relatedness cases (center row of **Figure 2*b***) are expected to sometimes behave like the zero-relatedness cases and sometimes like the high-relatedness cases, depending on the fitness costs and benefits of the allele that is under selection.

It is also conceivable that species B has no effect on species A and so no competitive or cooperative traits are favored (center column of **Figure 2*b***). Nevertheless, cells of species A may still express phenotypes that evolved for an entirely different purpose but accidentally affect species B positively or negatively, resulting in commensal or amensal fitness effects, respectively. Indeed, such accidental effects of A on B can occur even when species B affects the fitness of species A. Whenever the fitness effect of one species on the other is significant, however, natural selection may drive the emergence of true cooperative or competitive adaptations.

Unlike interactions among cells of one genotype, interactions with other strains and species are predicted to be able to evolve to be either positive or negative in their fitness effects. Competitive adaptations—either exploitative competition (adaptations that limit the available resources) or interference competition (adaptations that directly harm others)—are expected to dominate whenever species share limiting resources. The conditions for the evolution of cooperative interactions are more restrictive and require not only that the second species is growth promoting but also that there is high relatedness in the first species. Moreover, growth promotion may not be the norm in the densely packed and nutrient-limited conditions of many microbial groups. The basic theory then appears consistent with the genotypic view that marks a clear boundary between interactions within genotypes and between different genotypes, be they different strains or different species.

HELPING ENEMIES, HARMING FRIENDS

Our predictions on interactions between genotypes focus on whether genotypes evolve to have a net positive or net negative fitness effect on one another. This leads to the key predictions that cooperative adaptations are most likely to emerge between two genotypes that are already benefiting one another. Conversely, strong resource competition tends to generate competitive phenotypes, such as toxin production. Although these predictions hold for the overall fitness effects, cooperative adaptations can also occur between competitors, and competitive adaptations can occur between otherwise cooperating pairs. For example, if a toxin made by species A causes species B to secrete its own toxin in response (15), then natural selection could, in principle, cause species A to produce an enzyme that moderates the impact of its own toxin. Formally, this new trait would be a cooperative adaptation, albeit one that is embedded in a net competitive interaction. Similarly, one can imagine that competitive traits can influence the equilibrium fitness effects in a net cooperative interaction. We do not discuss such cases further here, but note that they are an interesting and potentially important component of the evolution of microbial interactions.

What Determines Relatedness and Ecological Competition with Other Genotypes?

Our review of the theory suggests that the emergence of cooperative versus competitive interactions depends on two key variables. The first is the frequency of cells of the same genotype (relatedness), and the second is the fitness effect (ecological competition) of foreign genotypes on a focal genotype. But what factors determine these key variables in natural microbial communities? A key factor influencing relatedness and ecological competition is cell density. A general effect is that low overall cell density reduces the impact of any interactions.

Commensalism: genotype A increases fitness of genotype B, but B has negligible impact on A. The effect of A on B is accidental, i.e., not an evolutionary adaptation

Amensalism: genotype A reduces fitness of genotype B, but B has negligible impact on A. The effect of A on B is accidental, i.e., not an evolutionary adaptation

This can limit natural selection for both competitive and cooperative phenotypes (13). However, many microbial communities, particularly surface-attached communities such as biofilms, achieve high cell densities in which interactions are both common and strong (43, 87, 97). We focus our discussion on biofilm-like conditions.

Relatedness. Most simply, relatedness (defined in the sidebar Inclusive Fitness Theory and Relatedness and **Figure 2**; redefined for HGT in the sidebar Horizontal Gene Transfer) depends on whether a cell is surrounded by clone-mates at the scale at which a particular social trait operates. A key predictor of this is the frequency at which new genotypes migrate in and colonize a particular environment (57). This rate should be considered relative to the rate at which cells divide in a community because cell division can lead to the loss of slower-growing genotypes and genetic diversity. Indeed, if a particular genotype can eliminate competitors, relatedness will increase and promote cooperation both within clonal groups of cells and between cooperating strains (89, 94, 131). However, cell division also increases the potential for loss-of-function mutants in cooperative traits, which can act in the opposite way and undermine relatedness-based cooperation (49, 108).

Perhaps the most important effect of cell division on relatedness is the creation of clonal patches of cells. Although genotypic segregation is determined by many factors, it is particularly likely when nutrients are limited. Intuitively, this occurs because nutrient limitation prevents most cells from dividing. This drives population bottlenecks that reduce genetic diversity (24, 44, 66) (**Figure 1*d***). Our simulation models have shown how these population bottlenecks can promote cooperation within genotypes by preventing cooperator genotypes from helping competing cheater genotypes (**Figure 3*a***) (85, 95). An elegant empirical paper has since demonstrated that these processes play out as expected in colonies of the budding yeast *Saccharomyces cerevisiae* (140). In diverse communities, the presence of additional species that do not benefit from the cooperative phenotype can further insulate cooperators from cheater genotypes (10, 85).

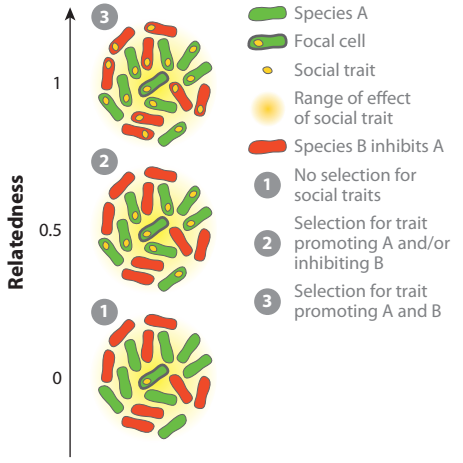

Figure 3

Horizontal gene transfer (HGT) makes it possible for a focal cell to give a social trait to a distantly related species. Relatedness approximates to the probability that cells affected by the social trait are the same genotype, i.e., carrying the social trait. We assume that the two species compete for resources and are both affected by the social trait (other interesting assumptions are possible but are not dealt with). We consider three scenarios. 1. HGT has little effect and only the focal cell carries the social trait. Relatedness is, therefore, low, and the social trait is likely to be disadvantageous. 2. HGT moves the trait within species A, which generates a case of high within-species relatedness. Traits that benefit carriers of the trait while harming the other genotype are favored. 3. HGT moves the trait between all cells. The two species are now related at the focal social trait and cooperation is favored at that locus.

Microbes can also actively influence their own spatiogenetic structure. For example, motility tends to disrupt spatiogenetic structure, as seen during swarming and biofilm formation in *Pseudomonas aeruginosa* (20, 61). However, twitching motility mutants in *P. aeruginosa* biofilms spatially segregate from motile wild-type cells, showing that the opposite effect can also occur (60). Motility has also been shown to be critical for the outcome of ecological competition in *P. aeruginosa* and *Agrobacterium tumefaciens* biofilms (2).

Spatiogenetic structure: the degree of assortment of cells by their genotype in space

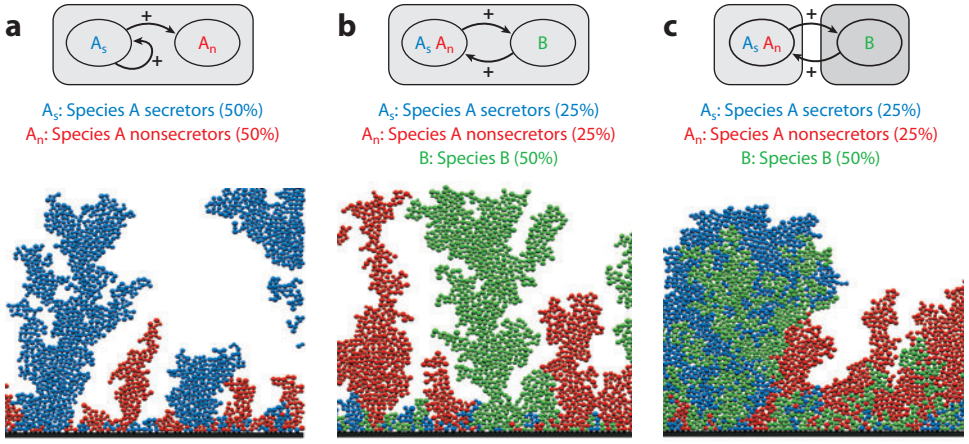

Figure 4

Computer simulations of bacterial biofilms. (*a*) Two competing strains of a single species emerge from a mixed layer of cells inoculated on a surface to form towers of clonal groups. As a consequence of high relatedness in these towers, cooperative secretors (*blue*) can outgrow nonsecretor competitors (*red*). Adapted from Reference 95. (*b*) In between-species cooperation, spatial segregation due to nutrient competition between all cells prevents the two secretor genotypes from mixing and benefiting from each other and selects against the genotype of species A carrying the costly cooperative trait (*blue*). (*c*) However, if the two species have different limiting nutrients (**Figure 2*b***, top right), the two secretors can then mix, and species A cooperators are favored. Adapted from Reference 85.

Bacterial phenotypes that affect adhesion to other cells and abiotic surfaces can also influence genetic mixing. Cells of the budding yeast *S. cerevisiae* stick to each other under high density and flocculate to form a protective clump of cells. This is driven by possession of a particular glycoprotein, and cells that make the glycoprotein preferentially stick to cells that also have it, thereby excluding those that do not express the relevant locus (134). Microbes can also attach to foreign genotypes (**Figure 1*b***), which decreases genetic similarity, as is seen in the complex patterns of coaggregation between dental species (114). Indeed, coaggregation may be central to promoting cooperation between genotypes as is discussed in the empirical data section on cooperation below.

The emergence of spatiogenetic structure is expected to promote cooperation at the spatial scale of the resulting single-genotype patches (65, 68, 95, 146). However, the effect on cooperation between different genotypes is not as simple as **Figure 2** suggests (85). This is because spatiogenetic structure can keep genotypes apart that have the potential to cooperate, thereby preventing them from helping one another (**Figure 4*b***). The strength of this effect, however, depends on the relative importance of nutrient competition between genotypes compared with the benefits they provide for each other (13, 58, 59, 86). Indeed, in the extreme case that there is no nutrient competition between two genotypes, but there are mutually beneficial secretions, the two genotypes can interdigitate and effectively cooperate (85, 86) (**Figure 4*c***).

Ecological competition. Strong ecological competition between different genotypes limits the potential for the evolution of cooperative traits (**Figure 2*b***). Moreover, as discussed above, strong nutrient gradients can drive spatiogenetic structure that allows cooperation within clonal groups but may limit interactions between different genotypes that might otherwise cooperate (**Figure 4*a*,*b***). What then determines the strength and impact of ecological competition between cells in a given microbial

community? High availability of resources can reduce ecological competition between cells (34, 95) and can occur not only when resource concentration is high but also when diffusion and flow rates are high or when cell growth rate is low. Even when nutrients are relatively abundant, however, cell division often eventually leads to dense communities with nutrient gradients. Theory and experiments have predicted and verified the existence of these gradients under a wide range of conditions (95, 110, 147, 162), which means that nutrient competition is strong at least at some points in the community (24, 61, 95).

The phylogenetic similarity of genotypes in a patch is also likely to influence the strength of ecological competition because distant genotypes, with possibly distinct metabolisms, tend to compete less for common resources, as has been shown empirically (33, 55, 84). Accordingly, ecological competition is expected to drive either the evolution of niche separation between genotypes (48, 127) or the extinction of one of the genotypes. Such niche separation has emerged in evolutionary experiments conducted in *Escherichia coli* (70, 121) and *Pseudomonas fluorescens* (109). Similarly, in biofilm experiments using *Burkholderia cenocepacia*, populations evolved into multiple distinct, spatially separated cell types after approximately 1,500 generations (107).

A final key factor affecting the impact of ecological competition is population demography. Cooperative phenotypes can sometimes allow a genotype to win outright within a microbial group. However, it is also possible that a cooperative phenotype can lose locally but win globally if the phenotype leads to large gains in local productivity (6, 12, 41). However, this depends on cooperation being able to drive local expansion and dispersal to other groups. For this reason, factors, such as frequent disturbances, that allow groups to expand and colonize new patches can promote cooperation over competition (9, 119, 123). Simulations suggest that the effects of local population expansion and migration can favor communities that alter their local environment toward optimal conditions for growth, which reduces nutrient competition between genotypes (157).

In sum, the emergence of spatiogenetic structure is likely to be key in promoting cooperation within clonal genotypes in microbial communities. Cooperation between different strains or species, however, appears more difficult to achieve and often rests upon high relatedness within each cooperating partner. Furthermore, between-genotype cooperation typically requires enough niche (and perhaps spatial) separation to prevent strong ecological competition but not so much as to prevent the reliable exchange of resources.

Ecological stability. Our predictions so far have all been based on whether cooperative or competitive interactions are likely to evolve. However, there is a second important question: Given that a cooperative or competitive interaction does evolve, will this lead to a stable community or will some genotypes go extinct? The drivers of ecological stability receive attention in conservation biology, where it is important to know whether losing one species leads to losing many more (4, 88, 135). A key model by May (79) predicts that more complex ecosystems are less stable, where complexity is increased by any one of the following: species richness, interaction strength (expected to correlate with the extent of cooperative and competitive interactions), and connectance (the number of species each species interacts with). These predictions were broadly supported by experiments with laboratory microcosms (31). However, the relationship between diversity and stability is not simple (135). If increasing diversity results in large decreases in interaction strength, then more diverse ecosystems can be more stable (82). There is also evidence that diverse ecosystems are better at resisting invasion by new species, including in gut bacteria (23, 141).

In addition to complexity, another factor that affects coexistence is spatiogenetic structure. Experiments in which three *E. coli* populations were grown together showed that an intermediate level of spatial segregation was necessary for a rock-paper-scissors interaction

that maintains all strains, presumably because segregation reduced the strength of ecological competition (58). Similar results were found in laboratory experiments with a three-species community of soil bacteria (59). In both studies, the community collapsed if grown in well-mixed liquid culture because the strongest competitors under those conditions would take over (58, 59). Another factor that can affect coexistence is the colonization order and timing of different genotypes (35, 67).

Can we then predict whether competitive or cooperative interactions are more likely to promote the persistence of the genotypes involved? The answer does not yet seem clear. Instability in May's model is associated with positive feedbacks that can come from cooperative interactions whereby multiple species all help one another and increase in number indefinitely. This result seems to suggest that cooperative interactions are less likely to be stable, something also noted in simple two-species models of cooperation (80). Other models suggest that cooperation can be stable (118) and that positive interactions are in fact more likely to persist over time, as they keep populations above the extinction threshold (54, 157). Such effects are particularly important to consider as they work against the genotypic view of limited cooperation between genotypes. One pattern that does seem to be both predicted and empirically supported is that a majority of weak interactions is important for ecological stability (88). Stability criteria, therefore, may tend to favor zero interactions or weak commensal and amensal interactions over strongly cooperative or competitive adaptations. Nevertheless, more work is needed before we can determine what types of interactions will maximize community stability.

EMPIRICAL DATA ON COOPERATION AND COMPETITION IN MICROBIAL COMMUNITIES

Despite the complexities, we believe that the theory can be reduced to our simple heuristic—the genotypic view—which we propose as the null model for microbial interactions. But what is the evidence for the primacy of cooperation within genotypes and competition between genotypes? Evidence for cooperation within genotypes is already strong and discussed extensively elsewhere (96, 156). We therefore focus here on interactions between genetically different strains and species. We start with examples of specific pair-wise interactions between different genotypes before moving on to studies that have attempted to survey interactions across large numbers of strains and species.

Cooperation

There are a number of good recent reviews that discuss case studies of cooperation between different strains and species, and show that cooperation between microbial species certainly can occur (69, 73, 128, 142). Some of these case studies come from pairs or a few species grown under seminatural conditions to infer that cooperation is likely to occur in the natural setting. The most striking examples of cooperation are cases in which the different genotypes are coinherited between generations, which is a powerful mechanism to align the evolutionary interests of different genotypes (30). The extreme illustrations of this process are lichens, which contain a fungus and either algae or cyanobacteria, and the origin of the eukaryotic cell. In this sense then, cooperation between microbes has been pivotal in the history of life and should certainly never be considered unimportant. But we are interested here in interactions between free-living species. Here too, one sees evidence of the importance of close interactions for cooperation. One example is the two-species aggregate *Chlorochromatium aggregatum* (**Figure 1*b***), in which nonmotile, photosynthetic sulfur bacteria attach to motile β-proteobacteria, providing them with fixed organic carbon in exchange for a ride toward sulfide-rich areas (101). However, because of difficulties in cultivating the aggregate or its individual species, it remains unclear whether the interaction does

indeed result in overall fitness gains for both parties (101). Attachment also occurs between the bacterium *Pelotomaculum thermopropionicum* and the methanogenic archeaon *Methanothermobacter thermautotrophicus*, whereby *P. thermopropionicum* uses its flagella to attach to *M. thermautotrophicus*, with flagella attachment inducing the latter to exchange metabolic services with the former (130). This example illustrates some key elements that are likely to be important for a cooperative interaction to stably emerge between species. First, the two species have very different metabolisms, which limits ecological competition between them. Second, their metabolism is so different that one species can live off of the hydrogen produced as waste by the other. Finally, the two species are physically attached to one another and respond to each other metabolically, which suggests that anything that a focal cell does to the other genotype is likely to feed back on itself (30). The prevalence of physical attachment in all of these examples may suggest that it is a particularly important component of between-genotype cooperation. Consistent with a role for attachment in cooperation, there are sets of dental bacteria that depend on each other to attach and grow on a saliva-conditioned surface (104, 105).

A second class of examples of cooperation is strains that are genetically engineered or artificially selected. The engineered examples include pairs of auxotrophic mutants in *E. coli* and *S. cerevisiae*, where each strain is unable to make a particular amino acid but can exchange it with the other strain (131, 158). Experimental evolution can also promote the potential for cooperation between different strains and species (47, 52, 131). These experimental studies are valuable, as they can directly test the conditions required for cooperation between strains. For example, Harcombe supported the theoretical predictions that reciprocal benefits and spatial structure can each promote cooperation between species (47).

Competition

There are many examples of competitive adaptations in one genotype that harm other strains and species. These come in two broad categories: adaptations that limit available resources (exploitative competition) and adaptations that directly harm other strains and species (interference competition). Nutrient uptake is necessary whether or not a strain is surrounded by competing genotypes, so not all nutrient uptake is an adaptation to take nutrients before neighbors can get them. However, there is evidence that microbes use rapid but inefficient metabolism to compete. In particular, it has been argued that the common use of fermentation when oxygen is present is a mechanism to generate a high growth rate but with a resulting low yield (106). Experiments with both *E. coli* and yeast support this idea (75, 145).

Competitive adaptations that actively harm other genotypes include the secretion of polymers that can push a genotype up and out and suffocate others (92, 160, 162). Smothering phenotypes can also emerge when one species evolves to better use nutrients provided by another species, which was seen during experimental evolution of *Pseudomonas putida* with *Acinetobacter* sp. (46). However, the clearest examples of harming adaptations that suggest different genotypes are often competing are antimicrobials.

Many species possess either broad spectrum antibiotics or bacteriocins that target phylogenetically similar strains (15, 51, 115–117). The abundance of bacteriocins may be because ecological competition is most likely between phylogenetically close genotypes that tend to occupy the same niche. The importance of antibiotics in bacterial evolution is underlined by evidence for ancient resistance mechanisms that predate anthropogenic use of antibiotics (21). Such coevolution is nicely illustrated by *Streptomyces clavuligerus*, which produces cephamycin C, a β-lactam antibiotic. Many species, including *E. coli*, *Staphylococcus aureus*, and others, produce β-lactamases, which confer resistance to the antibiotic. However, *S. clavuligerus* also produces clavulanic acid that binds to β-lactamase and inactivates it (72, 112). Because secreted antimicrobials are costly to the focal cell while helping all nonaffected cells in the

neighborhood (spite; see sidebar, Inclusive Fitness Theory and Relatedness), natural selection for toxins is similar to many potential forms of cooperation between genotypes (37). This is particularly clear in bacteriocins that appear to require cell lysis to be released, such as colicins and pyocins. Unlike rapid and inefficient metabolism, suicidal toxin secretion relies on the presence of both clone-mates and competitors for natural selection to favor their use (**Figure 2*b***) (37).

In addition to secreted toxins, there are also contact-based toxins that can be injected into (type VI secretion) (53) or placed onto (contact-dependent inhibition) (3) neighboring cells. Another potentially competitive strategy is the disruption of quorum sensing through the secretion of degradative enzymes. For example, *Streptococcus mutans* uses quorum sensing to regulate the production of bacteriocins that inhibit the growth of *Streptococcus gordonii*, a coinhabitant of the human oral cavity. *S. gordonii* degrades the quorum-sensing peptides of *S. mutans* and thereby inhibits its bacteriocin production (149).

Finally, a recent proposal contends that the effects of competitive evolution in bacteria are also seen in their stress responses (15). In particular, many of the bacterial stress responses have the ability to detect the two main classes of ecological competition: exploitative competition and interference competition. These map onto responses to nutrient limitation—stringent response, general stress response—and responses to cell damage, such as the SOS response and envelope stress responses. The evidence that these stress responses are indeed functioning in competition sensing comes from the fact that they are frequently used to upregulate bacteriocins and other antibiotics (see sidebar, Helping Enemies, Harming Friends), whereas it is very rare to see such inductions in response to more-typically abiotic factors, such as heat and osmotic stress.

Accidental Effects

Not all positive or negative effects of a focal genotype on another will have evolved because it has those effects on the second genotype. That is, organisms are constantly adapting to their surroundings in ways that may happen to have secondary positive or negative impacts on the fitness of other genotypes. For example, waste products in a focal species can be a resource for another species (128, 159). Such interactions are formally not evolutionary adaptations of the focal genotype to the other genotype if they have not been shaped by feedback effects from the recipients to the focal genotype. Key examples are commensal or amensal interactions in which there is no fitness effect in one direction between two genotypes. Commensalism—one-way positive effects—is typically emphasized over amensalism in the literature (69, 73, 128), although it is not clear that it is actually more common in practice.

We do not know how important commensalism and amensalism, and more generally, nonadaptive interactions are in nature. However, natural selection is not always strong in bacterial populations growing on surfaces (44, 66), and low abundance genotypes tend to exert weak fitness feedback effects on high abundance genotypes. In addition, several examples are commonly interpreted as one species accidentally helping another, such as when one genotype feeds on the waste products of another (128), something seen to emerge in the experimental evolution of both *E. coli* (121) and *B. cenocepacia* (107). Furthermore, a set of *Vibrionaceae* genotypes contained siderophore producers and nonproducers, with, importantly, nonproducers retaining the ability to take up the siderophores of the producer strains (13).

It has also been suggested that many bacterial species are currently not culturable in the laboratory because they require secretions by other strains to grow (25, 26). D'Onofrio et al. (25) found that some genotypes isolated from sand particles could only grow in the presence of other siderophore-producing genotypes. In addition, Ernebjerg & Kishony (26) found that soil samples diluted and grown on agar showed a disproportionate increase in growth rate, with an increasing number of bacterial strains per plate, consistent with the finding that some

genotypes produce metabolites that promote the growth of others. Further increases to cell density, however, reduced growth rate, suggesting that ecological competition may dominate at high densities (26).

The idea that microbes will lose phenotypes that are provided by other genotypes has recently been developed into the Black Queen hypothesis. The Black Queen hypothesis states that in any one environment, most species will lose genes for "leaky" phenotypes, i.e., for cooperative traits whose benefits they can get reliably from other genotypes (91). For example, *Prochlorococcus* strains have lost the KatG enzyme that protects against reactive oxygen species and appear to rely on other microbial species that have retained this ability (90). Although we have discussed the Black Queen under accidental effects, the underlying process is very similar to the evolution of cheaters within a genotype. The distinction is that the Black Queen hypothesis does not predict that receiver genotypes have a negative (cheating) effect. There may be no impact of the receiver genotypes or even positive reciprocation in other "leaky" goods that could drive the emergence of networks of cooperating genotypes (125).

The latter proposal is particularly important for our discussion, as it works against the predictions of our genotypic view. Not all studies assess the effects of recipient genotypes on donor genotypes (13, 25), but there appears to be evidence for both competitive and cooperative feedbacks. Interactions between evolved strains in the *B. cenocepacia* study above are more consistent with a producer-exploiter relationship than commensalism (107). In contrast, coculture of a *Prochlorococcus* sp. and an *Alteromonas* sp. increases cell numbers of both species relative to monoculture (90).

The Black Queen hypothesis deserves more investigation. It also raises an important complexity discussed in the evolutionary literature between evolved dependence and the canonical view of cooperative adaptation between genotypes (81). A host can evolve to rely on a chronic parasite for certain factors that it produces, such that if the parasite is removed, the host suffers. Such cases are difficult to distinguish from evolved cooperation but have different evolutionary origins and properties.

Community Assessments of Interactions

The above examples show the potential for both cooperation and competition. Estimating their relative frequency, however, needs studies that attempt to look at large numbers of interactions between genotypes in as unbiased a manner as possible. There are still relatively few relevant studies and, moreover, the intention of most such studies is not to estimate the net fitness effects of each genotype on each other genotype. A recent collaboration of our laboratory with Thomas Bell's attempted such an estimate in species isolated from two communities: a tree-hole community and a marine community (28). Results suggested that the large majority of pair-wise species interactions were competitive in the sense that at least one of the two species suffered reduced productivity in coculture relative to monoculture (see Inferring Interactions below; **Figure 5*a***).

The conclusion of competition being dominant is consistent with data from other study systems, which suggest that at most 14% of interactions between strains or species are cooperative (25, 28, 113, 143). A study on *Streptomyces* communities is particularly interesting because strains extracted from the same grain of soil tended to be more likely to reciprocate the effect—positive or negative—of its partners, which is consistent with the view that strains that commonly meet drive adaptation in one another (143). Furthermore, a study of 118 species inferred pair-wise interactions by building a metabolic model for each species on the basis of metagenomic data and species distribution patterns (34). This again found that the majority of interactions were of a competitive nature, although commensal—and rarely mutualistic—interactions could be favored under specific environmental conditions (34). One partial exception is a computational study of seven species, which suggested that commensalism is typical,

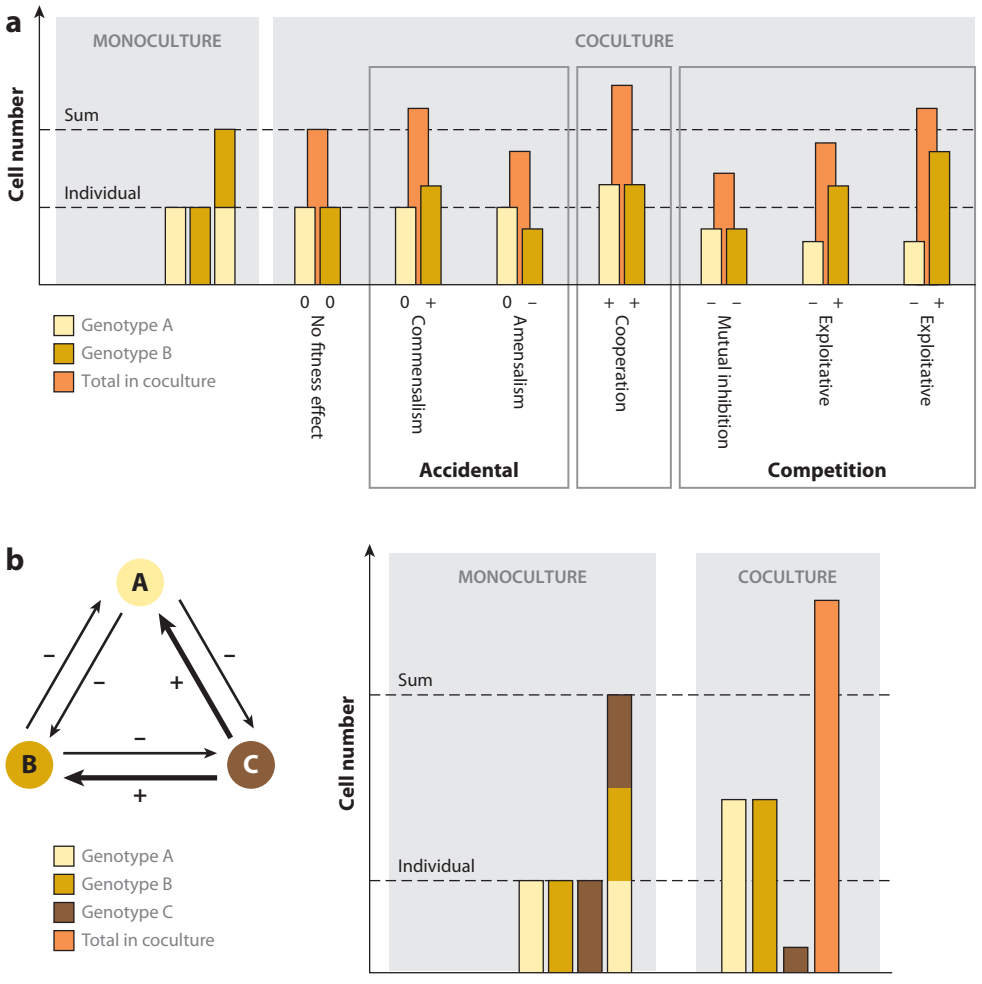

Figure 5

A first assessment of social interactions from growth in mono- versus coculture. (*a*) To classify social interactions between two strains or species, a hypothetical experimenter first grows each genotype in monoculture and then repeats the experiment (same conditions and resources) with the two genotypes growing in coculture (same initial cell number for each strain, twice as many in total). The plot shows all possible outcomes of such an experiment and the types of interactions that might be inferred from them. The interaction is only most consistent with cooperative adaptation if both genotypes increase with respect to their final number in monoculture (*bottom dashed line*). It is most consistent with competitive adaptation if one of the two genotypes decreases and the other increases or decreases compared to monoculture. If the experimenter cannot distinguish between the final numbers grown in mono- and coculture of one of the two genotypes, this is consistent with the effects on the other genotype being an accidental effect that was not selected for that purpose. Note that an increase in total cell number (*orange bars with respect to top dashed line*) does not necessarily indicate a cooperative interaction. Detailed mechanistic analysis is required to refine these first classifications. (*b*) Strong synergistic effects of coculture do not necessarily imply cooperation between genotypes. A hypothetical three-species network is shown in which one species, C, provides a limiting nutrient to two other species that harm C and each other.

although this deliberately focused on diverse species in which ecological competition is less likely (63).

Data from pair-wise interactions tend to suggest that competition is most common. Scaling up these pair-wise interactions into a full network of interactions has the potential to lead to positive feedback loops of cooperation between sets of species that dominate the community (135). Total productivity does tend to increase with more genotypes in coculture, but this increase is modest and still consistent with the majority of genotypes doing worse in coculture than when growing on their own (28, 55). Moreover, even in cases in which the majority of genotypes strongly benefit from being in coculture, it can still be that all interactions are noncooperative. For example, consider one genotype that fixes nitrogen and releases enough organic nitrogen to stop two other genotypes from being growth-limited by nitrogen. The latter two genotypes may now grow extremely well and end up harming the first genotype (**Figure 5*b***).

What drives the modest increase in productivity with diversity in competitive communities? The most likely explanation is that the addition of genotypes leads to a better coverage of all niches in a given environment (5). High diversity, therefore, can be a good thing for an engineering goal of increasing productivity, but improving cooperativity on top of this is likely to be key to generating highly productive communities. The current data support our use of the genotypic view as a null model of microbial interactions, but they are in many ways preliminary (28). Only a few sets of species have been studied, and there may yet be communities in which cooperation is dominant, which may be particularly likely in symbiotic communities, such as in the gut, where hosts may manipulate microbes to promote cooperation (129). Another reason for caution is that—with the exception of a computational study (34)—these studies rely on genotypes being culturable, which reduces the community to a subset of its members and may bias conclusions toward overestimating the role of competition. In sum, although we expect that competition is indeed the norm between genotypes, more work is needed before this can be concluded.

INFERRING INTERACTIONS

In this final section, we discuss approaches to dissect and understand microbial interactions. We focus on the study of interactions between different strains and species in which the classification of social interactions is challenging. We direct the reader to other reviews for the study of cooperative interactions involving identical genotypes (93, 96, 153) (see sidebar, Inclusive Fitness Theory and Relatedness).

Growth Comparisons in the Laboratory

A first proxy to classify interactions between two strains or species is to compare the productivity of each genotype growing on its own with the productivity of the two genotypes in a 1:1 coculture (**Figure 5*a***). Such experiments assume that productivity measures, such as final cell number, are a good estimate of evolutionary fitness. This is not always the case. First of all, growth rate rather than yield may be the key determinant of fitness in some ecologies. In addition, there are examples, such as σ^S mutants in *E. coli*, that outcompete wild-type cells in coculture but have reduced resistance to stress, which may mean that wild-type cells are the fitter genotype (145).

Such caveats aside, what does it look like when genotype A cooperates with genotype B? The first and obvious criterion is that the addition of genotype A increases the productivity of genotype B. However, if this is the result of cooperative adaptations in genotype A, we also expect that the productivity of A increases in the presence of B. This is because natural selection is not expected to favor cooperative adaptations that improve the overall fitness of a harmful foreign strain. Both genotypes benefiting in coculture, therefore, suggests that cooperative adaptation is dominant. If one gains and the other species loses—and we are indeed

observing the two genotypes in their natural interaction—the expectation is that one genotype has competitive adaptations that help it but harm the other genotype, analogous to those seen in predator-prey interactions. Finally, the other major category is that both genotypes do worse in coculture than when alone. This is consistent with a central role for the evolution of competitive adaptations in both genotypes that benefit the focal genotype but harm the other party. If growth in mono- and coculture cannot be distinguished in either of the two genotypes, one can conclude that there is no net fitness effect on each other for the studied environment. This could be, for example, because the two genotypes occupy different niches and do not interact.

As discussed above, there is also the potential for accidental effects by which one genotype helps or harms another genotype with adaptations that have nothing to do with competition or cooperation with the other genotype. Such by-products can potentially occur in any category but are most consistent with commensalism and amensalism, where fitness effects of genotype B on genotype A are not detectable. There is also considerable potential for accidental effects in an experimental sense whenever two or more genotypes are put together outside of the natural context. Indeed, one may observe results consistent with competition or cooperation that are in fact due to phenotypes that evolved in the absence of strong interactions with other genotypes in the experiments. In addition, if culturable genotypes are less likely to rely on others to grow, then this could bias conclusions toward overestimating the role of competition. This emphasizes the value of studying interactions in realistic conditions, such as pathogens in hosts (124) or environmental species in simulated natural conditions (5, 28).

There are then a number of reasons why simple coculture experiments must be taken with caution. This notwithstanding, coculture is arguably the most important technique for classifying interactions between strains and species, as coculture experiments allow a direct estimate of the net fitness effects of one strain on another (28). Coculture can be complemented with species knockout experiments in which certain species are removed from a natural community using antibiotics or the like (5). Another useful basic technique is to grow cells on the spent media of other genotypes to isolate secreted factors that affect growth (143).

Genetics and Genomics

Although coculture and spent media experiments allow a first assessment of the nature of interactions between genotypes, a full characterization ultimately rests upon refined characterization of social phenotypes using the tools of biochemistry and molecular genetics (e.g., 152). However, this is difficult on a large scale and a complementary source of information is the ever-growing wealth of genomic data. One must be cautious, as we are far from being able to divide a given microbial genome into loci of social and nonsocial effects. One proxy that may be statistically informative is secreted and nonsecreted proteins (100). However, it is clear that many nonsecreted proteins have social functions and vice versa.

The potential for nonsecreted proteins to be central in social interactions is well illustrated by the use of genomic data to infer niche coverage (78) and metabolic interactions (11, 50, 120). As discussed, two computational studies have ingeniously used genome sequences to infer the potential for positive or negative metabolic interactions between different strains and species (34, 63). However, these tools are not yet able to capture the effects of secretions that do not function primarily as metabolic substrates. Two important examples are siderophores and antibiotics, which have been the basis for studies that combine ecological and genomic data with experimental studies of social interactions between *Vibrionaceae* strains isolated from a single location (13, 14). One of these examples, discussed above, found that strains tend to all have the loci for uptake of siderophores but only some have loci for production (13). The second (14) found

GFP: green fluorescent protein

FISH: fluorescent in situ hybridization

that antagonistic interactions occur mostly between strains from different populations because strains within populations were resistant to local antimicrobial secretions. A genomic analysis showed that within populations, only a minority of strains harbored genes coding for the production of a highly inhibitory antibiotic and that the presence or absence of these genes, rather than transcriptional regulation, predicted which strains were "superkillers."

Although the presence or absence of loci can be informative, so too can the sequence variation between the genomes of different strains. Such analyses reveal that the siderophore loci of *P. aeruginosa* (pyoverdine loci) constitute the most divergent region in the species' core genome (136), and this displays strong evidence for diversifying selection. Specifically, natural selection for functional change over time can be inferred from a high number of nonsynonymous changes relative to synonymous changes in the coding sequence (high *dN/dS* ratio) (132). On the basis of these data, it has been argued that selection for within-species diversity may either be driven by selection to defend against the stealing of ferripyoverdine complexes from producing strains or to escape susceptibility to bacteriocins produced by other strains, which are taken up through ferripyoverdine receptors (139).

Such examples illustrate how the wealth of genetic and genomic data can be used to extract complex networks of interactions within microbial communities. However, it is also notable that the clearest examples are those that combine genomic data with physiological and experimental data of relatively well-understood phenotypes, such as metabolic reactions, siderophores, and antimicrobials.

Spatial Organization

Theory and experiments suggest that the spatial arrangement of different genotypes in microbial groups is central to their interactions (96). For this reason, the analysis and manipulation of spatiogenetic structure is of considerable interest. And, critically, spatiogenetic structure needs to be studied at the spatial scale at which a social phenotype of interest functions (93). Spatiogenetic structure can be studied by genetically engineering different genotypes to express fluorophores, e.g., green fluorescent protein (GFP) (**Figure 1*d***), and for species that cannot be transformed, there is fluorescence in situ hybridization (FISH), which hybridizes fluorescent molecules to an existing community using probes that target different genotypes (**Figure 1*c***). A key question is the extent to which single genotypes cluster in space, where the existence of clonal patches of a single genotype suggests cooperation within that genotype at that spatial scale (assuming limited opportunity for the origin and spread of cheater mutants). But can one make similar inferences from spatiogenetic structures for the interactions between different strains and species?

At least two characteristics can inform the nature of interactions between different genotypes: the shape of any boundary between genotypes and the extent of mixing between genotypes. For example, looking down on a colony of mixed genotypes often reveals sectors of distinct genotypes (**Figure 1*d***). If the boundaries between these genotypes (in the direction of growth) are straight on average, this is consistent with equal fitness in the two genotypes (38, 44, 64) and the genotypes are subject to genetic drift only (66). However, if one genotype expands outward in the familiar fan shape of an advantageous mutation, one can infer that it has a higher evolutionary fitness than the other genotype (44, 64). At this scale then, one can identify which genotypes have a higher fitness. However, from spatial patterns alone, it is not possible to assess whether the genotypes are net competitive or cooperative overall. This is because cooperating genotypes that are helping each other to grow can experience differences in growth rate that would lead one to fan out.

It is similarly unclear whether genotypic mixing can be used as a proxy to classify interactions (65). There is some evidence that genotypic segregation correlates with ecological competition between genotypes, whereas well-mixed genotypes indicate cooperation

(42, 58, 59, 65, 86, 99, 126). However, this requires that social interactions are more important for spatial structure than other effects on the spatial structure, such as the availability of nutrients (95), the properties of the growth medium (e.g., its viscosity) (8, 99), the presence and nature of liquid flow, and the phenotypes of the cells in the biofilm (e.g., motility, stickiness, secretions). Moreover, a cheater genotype can be under natural selection to mix with a cooperator, whereas the cooperator is under natural selection to remain segregated, creating a conflict of interest whose outcome will be difficult to predict a priori (146).

In sum, strong spatiogenetic structure is broadly consistent with competition between genotypes and mixing with cooperation within genotypes. However, these are not hard rules, and more theoretical work is required to see if other spatial characteristics can be included to better refine the link between spatiogenetic structure and interactions (44, 65, 86). Without this, other techniques, such as productivity comparison in pure and mixed cultures, are required. This is even true of the strongest spatiogenetic indicator of competition—lines of inhibition—as theoretically there could be an exchange of cooperative traits at larger spatial scales.

CONCLUSIONS

An important long-term goal of studying microbial communities is the ability to manipulate them toward some desired state or to engineer novel communities with a given set of specifications. Imagine being able to take a sample from the gut of a patient suffering from inflammatory bowel disease or a soil sample from an agricultural site and advise on how to manipulate the resident microbial community in a way that increases its overall production of a certain metabolite or its stability in the face of environmental perturbations. To what extent can we engineer a community to digest toxic waste or to fabricate a synthetic product more efficiently than is typically done with undefined microbial consortia (151)? This ability to rationally manipulate and build microbial communities would have implications in fields ranging from medicine to climate change.

Although the idea of synthetic ecology is increasingly discussed (62), the vision of rationally engineering stable microbial communities is largely beyond the current state of the art. Any review of the literature shows that microbial communities are incredibly complex. From an evolutionary point of view, conflicts of interest exist at many levels: Not only do strains compete for limiting resources, but HGT also results in the cells themselves competing with their social genetic elements for their presence in future generations (see sidebar, Horizontal Gene Transfer). Which of the strains, species, or genetic elements manages to survive and remain abundant within a community depends heavily on environmental conditions. Not only can these conditions alter the strength and direction of selection, but the behavior of the cells resulting from selection can in turn alter environmental conditions, creating complex feedback loops that make disentangling them a daunting task.

Making sense of this complicated picture will rely on resolving three key challenges: (*a*) identifying the different types of interactions taking place within the community; (*b*) determining how changes to various key parameters, such as nutrient levels and genotypic mixing, alter selection for these interactions; and (*c*) predicting how the community will evolve and stabilize, if at all, in the long-term. If full rational design proves too difficult, there is the option of artificially selecting for desirable traits of a community under conditions that promote cooperation.

Although the semantics of social interactions are always debatable (155), the definitions we have used here (see sidebar, Inclusive Fitness Theory and Relatedness) are based on their evolutionary effects and are intended to allow the origin and prognosis of interactions to be easily understood. On the basis of these definitions and the results of large-scale studies of social interactions (28, 34, 143), it is likely that studies on cooperative interactions

are overrepresented in the literature relative to the natural world (77, 128, 159). If the genotypic view is correct and cooperation between genotypes is relatively rare in nature, there are implications for our own interactions with microbes. Although productivity shows modest increases with species diversity (5), increasing cooperation may be the key to large productivity gains in areas such as biofuel production and waste breakdown, assuming that cooperativity can be achieved alongside ecological stability (28, 135). An intriguing possibility is that we have evolved to already do this in our guts, where there is considerable potential for secretions to influence the cooperativity of microbes (129). However, even here, in our most intimate association with microbial communities, our understanding of interactions is extremely limited. The study of microbial interactions is only now starting in earnest.

SUMMARY POINTS

1. Characterizing and ultimately controlling multispecies microbial communities requires an understanding of the social interactions between cells.
2. The genotypic view of microbial interactions suggests that cells of the same genotype will cooperate, whereas different genotypes will typically compete; however, with high relatedness within each partner and low niche overlap, cooperation between different strains and species may emerge.
3. Relatedness and niche overlap depend upon ecological conditions, including emergent spatiogenetic structure and resource availability.
4. In support of the genotypic view, natural examples of mutually beneficial cooperation between genotypes appear rarer than examples of competitive phenotypes, such as the secretion of antimicrobials. This view is supported by large-scale studies involving many strains and species isolated from natural environments.
5. Classifying and identifying interactions is possible through comparisons of growth rates of genotypes in mono- and coculture, through molecular, genetic, and biochemical characterization, through the analysis of genomic data, and through analysis of the spatiogenetic structure of a microbial community.
6. Future work should move from classifying microbial interactions to understanding their effects on microbial communities and how to manipulate them.

FUTURE ISSUES

1. Theoretical work is needed to determine the stability of multispecies networks of cooperative and competitive interactions.
2. A more complete set of theoretical predictions on how HGT affects selection for different social traits and its importance will help to generalize current predictions.
3. There is a need for further large-scale studies of interactions in microbial communities to clarify the relative importance of cooperative and competitive traits in nature.
4. Developing methods to increase the culturability of microbes in the laboratory will be key to conducting thorough analyses of their interactions.

5. In collaboration with the engineering disciplines, new theories and experiments are necessary if we are to reliably construct, evolve, and control microbial communities for our own purposes.

DISCLOSURE STATEMENT

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Errata

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