

Evolutionary limits to cooperation in microbial communities

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Microbes produce many compounds that are costly to a focal cell but promote the survival and reproduction of neighboring cells. This observation has led to the suggestion that microbial strains and species will commonly cooperate by exchanging compounds. Here, we examine this idea with an ecoevolutionary model where microbes make multiple secretions, which can be exchanged among genotypes. We show that cooperation between genotypes only evolves under specific demographic regimes characterized by intermediate genetic mixing. The key constraint on cooperative exchanges is a loss of autonomy: strains become reliant on complementary genotypes that may not be reliably encountered. Moreover, the form of cooperation that we observe arises through mutual exploitation that is related to cheating and “Black Queen” evolution for a single secretion. A major corollary is that the evolution of cooperative exchanges reduces community productivity relative to an autonomous strain that makes everything it needs. This prediction finds support in recent work from synthetic communities. Overall, our work suggests that natural selection will often limit cooperative exchanges in microbial communities and that, when exchanges do occur, they can be an inefficient solution to group living.

Black Queen evolution | cooperation/exploitation | ecoevolutionary model | genetic mixing | microbial communities

‘Benefit-of-the-species’ arguments ... provide for the reader an escape from inner conflict, exacting nothing emotionally beyond what most of us learn to accept in childhood, that most forms of life exploit and prey on one another.

Hamilton, 1975 (1)

Microbes typically live in dense communities containing many strains and species. These genetically diverse societies are widespread and central to how microbes affect us, including examples such as the gut microbiome, polymicrobial infections, and communities vital to bioremediation and nutrient cycling (2, 3). In these collectives, ecological interactions are thought to be both common and strong given that cell density is typically high and that microbes possess many phenotypes that influence the reproduction and survival of surrounding cells (4, 5). Such social traits include many secretions, such as extracellular enzymes and scavenging molecules (4–6), and other beneficial “leaky” traits, such as detoxification agents (7) or amino acids (8, 9).

A central explanation for cooperative phenotypes in microbes is that they function to help cells of the same genotype (10, 11), which is backed up by a growing body of theory and experiments (12–18). However, it is also clear that, in nature, microbes commonly interact with many different genotypes (both different strains and species) in complex ecological networks (19–21). Do these different microbial genotypes cooperate with one another? Understanding this question is central to building models of microbial communities and how they will respond to both environmental and anthropogenic perturbations (22).

Studies involving genetically engineered (8, 9, 23, 24) and/or artificially selected communities (23, 25, 26) emphasize how easily cooperation between genotypes can be achieved in the laboratory. Additionally, there are a growing number of suggestions that

cooperation should commonly evolve between microbial strains and species (27–30). This view contrasts with empirical surveys of natural bacterial communities, which suggest that competitive interactions predominate over cooperative interactions (31). However, it has also been suggested that cooperation between different genotypes may explain the unculturability of many species in the laboratory when in monoculture (32–34). If correct, studies with culturable species could underestimate cooperativity in microbial communities.

The potential for cooperation between different microbial genotypes then remains unclear. Indeed, we even lack clear predictions of what to expect. There is a need for general theory on cooperation between microbial genotypes. One microbial interaction that has been explored theoretically is syntrophy, where one species produces a toxic waste product that another species consumes (35–38). Syntrophy is likely to be ecologically important and under some conditions (36), can benefit both species. However, syntrophic species need not pay energetic costs to interact: one species is producing waste, and the other species is feeding. Such byproduct cooperation can, therefore, readily evolve but is fundamentally different to the exchange of costly secretions (39, 40). Other models have analyzed when costly cooperation between species is expected in microbes and other organisms (35, 39, 41). However, these models assume that there is no opportunity for one partner to express the beneficial trait of the other. Although this constraint will sometimes occur, there is considerable functional overlap in the cooperative traits of microbial species (7). In addition, the potential for horizontal gene transfer in microbes means that there is a broad scope for a focal strain to pick up the phenotypes of co-occurring strains and species (42–44).

Significance

Microbes form dense and diverse communities that affect every aspect of our lives. Microbial communities are often viewed as cooperative networks with species working together toward a common goal. Here, we critically evaluate this view using an ecoevolutionary model. We show that cooperating with other species can be a poor evolutionary strategy, because it renders a cell dependent on species that may not be nearby. Moreover, when cooperative exchanges do evolve, they are inefficient and reduce the productivity of the community. Evolution by natural selection then limits the potential for productive cooperation between microbial species. We argue that understanding these limits and how to overcome them will be key to engineering microbial communities for our own ends.

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Here, we examine the potential for microbial cooperation between different strains and species. We base our work on the well-established models of within-genotype microbial cooperation for a single public good (12, 18, 45–47) so that the relationship to previous work is clear (*SI Materials and Methods*). We add one key feature to these models: we allow cells to invest in multiple distinct cooperative secretions, such that there is the potential for different genotypes to exchange secretions with one another. Our analysis shows that the degree of genetic mixing defines the potential for cooperation both within and between genotypes. Low mixing favors genotypes that produce all secretions, whereas high mixing favors genotypes that do not produce any at all. Only for intermediate levels of genetic mixing do we find between-genotype cooperation, where strains produce a subset of secretions and rely on other genotypes for the complementary traits. Moreover, the form of cooperation that emerges is inefficient and results in a loss of productivity relative to one genotype making all secretions. Natural selection limits both the occurrence and effectiveness of cooperation within microbial communities.

Results and Discussion

Logic of the Model. We are interested in the potential for the evolution of cooperative exchanges in microbial communities. Cooperative phenotypes can be central to growth in microbes (4, 6, 48) in both natural (49–51) and disease (52) settings. These phenotypes are typically costly to produce but provide large shared benefits that increase the yield of all cells in a neighborhood (4–6, 48). To reflect these strong benefits, we focus on secretions that are essential for bacterial growth and set final population yield (*Materials and Methods*), although our conclusions also hold for nonessential secretions (Fig. S1). Whereas our analysis is phrased in terms of secretions, like enzymes that liberate nutrients, it also captures a range of other leaky beneficial traits, such as detoxifying enzymes like catalase (7) or amino acids (8, 9).

Fig. 1 outlines the logic of our ecoevolutionary model. For two secretions, our model has four genotypes: a producer of both traits [1,1], a producer of the first trait [1,0], a producer of the second trait [0,1], and a nonproducer [0,0], where for simplicity, 1 and 0 denote production or no production, respectively, of a given secretion, thus labeling each genotype. Our modeling is intended to capture two related scenarios. The first scenario is a single strain that makes multiple secretions, where loss of function mutations can generate the above trait combinations. The second scenario is where the different genotypes come from

a combination of interacting strains and species that have similar ecologies. This latter case has clear potential for complexities that we do not represent, but we can, nevertheless, capture the evolutionary dynamics that would be driven by the focal traits. In addition to extending models of single secretions, our model is intended to formalize the hypothesis that cooperation can arise by different genotypes losing the genes for different secretions (29). This hypothesis was developed from the idea of “Black Queen” evolution (7). Specifically, if many strains all make and use the same leaky trait, some strains can lose the trait and rely on others to grow. For a single trait, Black Queen evolution then has similarities to the evolution of cheating, where a diverse network of species may be involved as either a producer or receiver of a particular trait. When microbes possess multiple leaky traits, it raises the possibility that two genotypes may reciprocally exchange beneficial traits when one loses one trait and one loses another trait (29). Linked to this scenario, a key assumption in our analysis is that it is possible for a cell to make all secretions if favored by natural selection (i.e., in a system relying on two secretions, for example, there is always the possibility of a [1,1] genotype). Although not always the case, this possibility seems likely to be common, because many secretion systems are associated with horizontal gene transfer (42, 44). However, we discuss later the effect of some secretions only being achievable by some genotypes.

Between-Genotype Cooperation Emerges with Intermediate Levels of Genotypic Mixing.

We are interested in understanding the effects of natural selection when microbes produce more than one fitness-promoting secretion. In particular, we are interested in whether strains will evolve to exchange secretions. Fig. 2 gives the final (steady-state) frequencies of each of the different secretion genotypes as a function of the degree of genotypic mixing. We include a model of a single secretion, which recapitulates the well-known cooperator–cheater dynamics where limited genotypic mixing (high relatedness) leads to the evolution of secretion, whereas high genotypic mixing (low relatedness) leads to a loss of secretion (15, 16, 18, 45–47). What happens when cells can produce two secretions? At low mixing, the genotype that produces all secretions dominates, and at high mixing, one sees the opposite (the genotype that does not produce any secretions dominates). Interestingly, however, at intermediate levels of genotypic mixing, there is natural selection for partial secretors that produce only a subset of the secretions, such as [1,0], which rely on the complementary strain [0,1] for the other secretion. The emergence of such cross-feeding between partial secreting genotypes represents a simple form of between-genotype cooperation.

Why do complementary genotypes dominate nonsecretors at intermediate genotypic mixing? The answer is in the demographics of the system and the potential for something known as Simpson’s Paradox (45, 47). Within any one group, nonsecretors outcompete secretor genotypes because of the costs associated with making the secretions. However, the groups containing more secretors produce more cells in total to seed new groups. Then, at intermediate levels of genetic mixing, complementary genotypes are associated with more productive groups relative to nonsecretors and therefore, can outcompete them. Additionally, because there is a significant amount of mixing, one partial secretor genotype [0,1] will often meet the partner genotype [1,0] within a given group, allowing them to outcompete full secretors [1,1] that grow more slowly to make both traits. Partial secretors compete best against full secretors [1,1] when the costs of secretions are relatively high, because there is a more significant growth advantage from not making a secretion (Fig. 2).

The behavior of the model for two, three, and four secretions is broadly similar (Fig. 2 and Fig. S2). However, adding more secretions to the system increases the scope for cross-feeding genotypes to outcompete other genotypes. A key reason for this is that increasing the number of secretions increases the associated costs, which promotes cross-feeders over full secretors (Fig. 2). The importance of costs is shown by changing the number of secretions

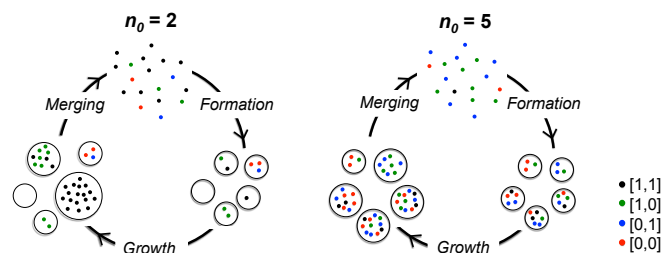


Fig. 1. Ecoevolutionary model of the microbial lifecycle. We considered three cyclically recurrent phases to model the evolutionary dynamics of a microbial population that depends on multiple secretions to grow: (i) formation (stochastic seeding of M separate subpopulations following a Poisson process with mean n_0), (ii) growth (independent evolution of each group following logistic growth, where the carrying capacity of the environment is proportional to the concentration of secretions in the focal group), and (iii) merging (M groups merge, and the cycle starts again after updating the genotypic frequencies). As an illustrative example, we show the effect of two different n_0 values in a two-trait model of four genotypes ([1,1], [1,0], [0,1], and [0,0], which make both secretions, one secretion, the other secretion, and none, respectively).

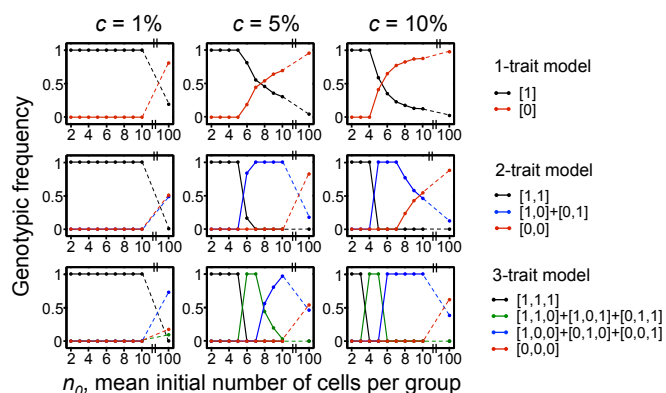


Fig. 2. The evolution of within- and between-genotype cooperation in microbial communities. We show steady-state frequencies of different genotypes as a function of the average number of cells founding each group (n_0) and the cost of each trait (c) in systems that depend on one, two, and three secretions to grow. Genotypes are labeled with one and zero to show whether they invest or not in a given secretion; [1], [1, 1], and [1, 1, 1] have the advantage at low n_0 , whereas [0], [0, 0], and [0, 0, 0] have the advantage at high n_0 . Cross-feeding genotypes that cooperatively exchange secretions dominate at intermediate values of n_0 (a proxy for mixing among genotypes), particularly for secretions with high cost (c).

in the system while keeping the total cost of all secretions constant (Fig. S2). A general prediction then is that raising the costs of secretions, either through a single secretion that is very costly or the expression of many individually costly secretions, can favor cross-feeders relative to full secretors. As we show below, however, using many secretions also creates problems, because cross-feeders need to reliably find multiple partners to be successful. This dependence on multiple other genotypes makes cross-feeding with many secretions particularly fragile to factors that limit access to other genotypes (Fig. S3).

Our model assumes that all strains compete for the same niche. Although this assumption seems reasonable given the competition observed between strains and species from a common environment (31, 53), the extent of niche overlap will vary between different genotypes. We, therefore, decided to test the importance of complete niche overlap for our conclusions. We did this by modeling two sets of interacting strains. All strains can cooperate with one another, but only within a set do the strains compete. Between sets, there is no resource competition consistent with each set occupying a different niche. Importantly, this model shows the same qualitative behavior as our simpler single-niche model: between-genotype cooperation requires intermediate levels of genotypic mixing to evolve by natural selection (Fig. S4).

In sum, we find that between-genotype cooperation can evolve by natural selection, particularly with high costs to secretions and intermediate levels of mixing of microbial genotypes.

Between-Genotype Cooperation Is Associated with Reduced Group Productivity. The two-way exchanges of secretions that emerge in the model can be viewed in at least two different ways. The first way is cooperation for mutual benefit. Indeed, if two complementary strains or species were examined in a laboratory experiment, one would find that each cannot grow alone but together they grow well, a result that is commonly used to diagnose cooperation or mutualism (31, 54). However, another aspect of the interaction is competitive, where each partial secretor is acting as a cheater on full secretors and its complementary partner. Can one then quantify the impact of this competition? A simple but powerful way to assess the effects of competition is to look at overall group performance, which can be harmed by genotypes investing in competitive traits instead of cooperative traits that benefit the group (55).

Fig. 3 shows the evolved group productivities as a function of founder cell number (the degree of genotypic mixing). The emergence of cross-feeding exchanges with increased genetic mixing is associated with a loss of group productivity—a diagnostic of wasteful competition that is inefficient at the group level. The greater the potential for between-genotype cross-feeding, therefore, the lower the group performance relative to a single strain that makes all secretions. Accordingly, any factor that promotes cross-feeding exchanges, such as increasing the cost or number of secretions, leads to greater losses in group productivity. Why this reduction? The emergence of this type of cross-feeding is associated with a loss of genes for secretion in each partner. This loss means that less of the secretion will accumulate in a cross-feeding group relative to a group of cells that all make all secretions.

The model does not allow strains to modulate the amount of secretion that they make. If allowed, will cross-feeders compensate for their low productivity by increasing their investment in cooperation? We evaluated this in two ways. First, we extended our model to allow strains to invest in three different levels of secretion (0.5, 1, and 2). Second, we developed another model that allows strains to invest any amount in secretions by considering mutations that can change the level of investment (*Materials and Methods*). Rather than increasing group productivity, however, allowing strains to modulate secretion levels only makes things worse for cross-feeders (Fig. 4 and Fig. S5). The evolution of cross-feeding is now associated with even greater losses in group productivity, because cross-feeders evolve to invest less in each secretion than full secretors. In sum, the evolution of cross-feeding cooperation between microbial genotypes is associated with significant levels of competition and mutual exploitation.

The idea that a cooperative interaction between genotypes can arise by exploitation has a precedent in discussions of mutualism and host–parasite evolution (54). There it has been observed that a chronic parasite might produce a factor that a host evolves to depend upon. Under these conditions, removing a parasite can harm a host, although the original basis for the interaction was strongly negative for the host. These situations, like in our system, can be viewed either as competitive or cooperative depending on the comparison taken. Specifically, if an experimenter simply removes a partner, he or she will diagnose the relationship as cooperative. However, this diagnosis will hide the evolutionary history and the fact that the partnership originally evolved through competition and exploitation. This historical effect of competition is not merely a definitional issue; it predicts that the productivity of microbial communities relying on cross-feeding exchanges will be low relative to their full potential.

Constraints on the Evolution of Between-Genotype Cooperation. The evolution of cooperation between genotypes is predicted with relatively high costs to secretions and intermediate levels of

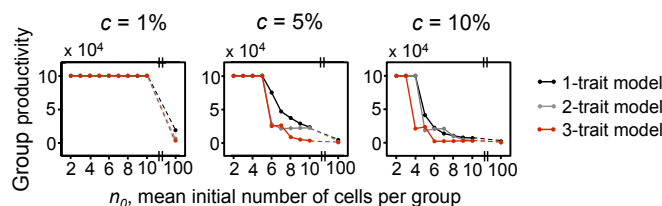


Fig. 3. Evolved average group productivity as a function of founder cell number (n_0). The emergence of cooperative exchanges between genotypes is associated with a loss of group productivity (two- and three-trait models). This loss of productivity occurs for the same reason that cheaters harm group function in single-secretion models (one-trait model). This outcome is particularly clear for secretions with high cost (c) and a high number of traits. Average group productivities were obtained from 1,000 independent subpopulations. SE bars are smaller than the plotting dots and therefore, not shown.

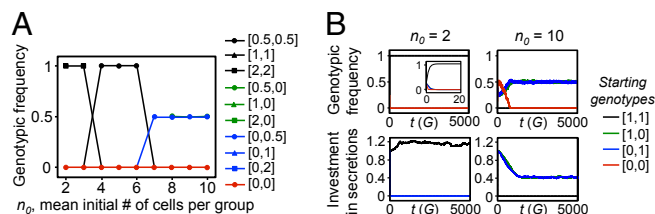


Fig. 4. Evolved investment into secretion production as a function of founder cell number (n_0). Allowing for differential investment in secretion production amplifies the tragedy of the commons, because cross-feeders evolve to invest less in secretions than strains that make all secretions. (A) Discrete model. Genotypes that invest two times as much in secretions ([2,2] instead of [1,1]) reach fixation when genetic mixing is low. However, at high mixing, the genotypes that fixate are cross-feeders that invest less in secretions than in the original model ([0.5,0] and [0,0.5] instead of [1,0] and [0,1], respectively). (B) Continuous model. If cells continuously tune their investments into secretion production, we find the same patterns, where cross-feeding strains invest less in cooperation than strains that make all secretions. *Upper* shows the genotypic frequencies of systems for low and high mixing, whereas *Lower* shows the mean investment in secretions. *Inset* clarifies that genotypic frequencies are identical at the beginning, but producers of both traits quickly reach fixation. SE bars for steady-state investments are smaller than the plotting lines and therefore, not shown. We set the cost per trait (c) at 5%.

mixing of microbial genotypes. Do these conditions commonly occur in nature? The fitness cost of secretion can be highly variable in strongly regulated traits, such as siderophores, depending on how much a cell makes (56). Nevertheless, estimates of costs of secretions are often low (in the range of a few percent of growth rate or less) (57–59). Low-cost secretions are expected whenever prudent regulation limits secretion to times when it is cheap to do so (59). The effects of costs are also limited when secretions have a private component that benefits a secreting cell more than other cells (60). If low-cost secretions are the norm in nature, then our model suggests that the emergence of between-genotype cooperation will be limited. Potential exceptions to this prediction are if cells use many relatively costly secretions (Fig. 2 and Fig. S2) or indeed, if using multiple secretions is, for some reason, disproportionately costly (Fig. S6).

What about the levels of genetic mixing in microbial communities? This key variable is still poorly understood (6, 22). However, what is clear is that many natural microbial communities display spatial structure, whereby microbes attach to surfaces and each other and grow (16, 61). This structuring will affect genetic mixing and is not accounted for in our model, which assumes that genetic mixing within any one microbial group is perfect. To examine the impacts of spatial structure, we implemented an individual-based version of our two-secretion model, where cells are seeded on a lattice and divide if they have space around them and access to both public goods (*Materials and Methods*). In particular, we focus on parameter ranges that strongly promote cross-feeding pairs in the absence of spatial structure within the group.

We first use the individual-based model to recapitulate the well-mixed model by allowing secretions to diffuse a long distance so that cells have access to all secretions. We then implement a low-diffusion scenario so that secretions only reach a limited range. This simple change has a strong qualitative effect on predictions: now, the genotype that produces both secretions is dominant (Fig. 5A). The reason for this effect is intuitive: spatial structure limits the potential for interactions with other genotypes. Cross-feeders are now much less likely to interact with their complementary partner, which means that they will often lack secretions that they need for their growth.

Spatial structure then tends to limit cooperation between microbial genotypes, which is consistent with recent empirical work showing that spatial structure can limit positive interactions between microbial genotypes (26, 62). This effect contrasts with

the typical conclusion that spatial structure promotes cooperation (13, 16, 17, 63, 64) by increasing the probability of interacting with the same genotype (10, 11). However, cooperation between genotypes in our multitrait model emerges under the same conditions as cheating in the classical models. Accordingly, we find that spatial structure simultaneously inhibits between-genotype cooperation and promotes within-genotype cooperation.

Another assumption of our model, which is important for genetic mixing, is that all interacting genotypes in the system make use of the secretions. This assumption is likely to be broken, because the extreme diversity associated with natural microbial communities suggests that there will often be many species present that do not use or make the secretions of our focal genotypes (65). In models of a single secretion, it has been shown that such passive genotypes can insulate a focal genotype from interacting with other genotypes that might use its secretions [social insulation (65)]. Fig. 5B shows the effect of introducing passive genotypes that do not use the secretions or resources of the focal group. Like the effect of introducing space, the presence of passive genotypes decreases the probability that cross-feeding genotypes interact with their complementary partner, thereby favoring strains that can function effectively in isolation. Put another way, passive genotypes reduce the group size of the focal interacting genotypes, which reduces the effective level of genetic mixing and promotes autonomous genotypes like [1,1].

We saw earlier that a high number of secretions can promote cross-feeding (Fig. 2 and Fig. S2). However, it is important to note that the introduction of passive genotypes has a disproportionately strong effect when cells use a high number of secretions (Fig. S3). With many secretions, each cross-feeder has to find multiple partners, and passive genotypes become more of a problem than when a cell has to find just one partner. In sum, the use of many traits presents cross-feeders with two problems. First, cross-feeding involving many traits is associated with greater reductions in group productivity than with fewer traits; communities become more inefficient (Fig. 3). Second, relying on many traits makes cross-feeding networks fragile in the sense that each cross-feeder will typically require multiple other genotypes nearby to grow (Fig. S3).

Although we expect the effect of passive genotypes to be strong, mechanisms that promote association between complementary strains will improve the prospects of cross-feeding. Candidate mechanisms that promote association include emergent sorting that can occur if two strains grow more in each other's presence than when alone (22, 65, 66), chemotaxis toward partner genotypes, or even direct attachment (67). The importance of such

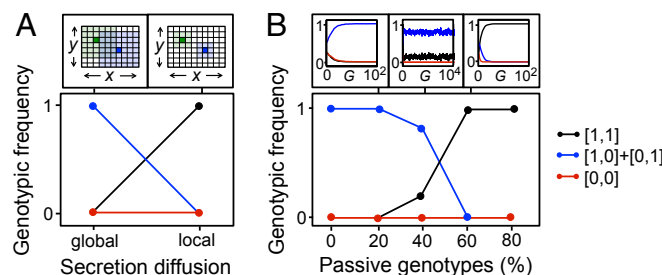


Fig. 5. Effect of spatial structure and social insulation on the evolution of between-genotype cooperation. (A) Spatial structure (individual-based model). Space hinders the evolution of between-genotype cooperation with local diffusion of secretions, because genotypes are prevented from interacting. *Upper* shows cartoons to illustrate the diffusibility of secretions. (B) Social insulation modeled in well-mixed groups. The presence of passive genotypes, which do not compete with focal genotypes and do not contribute with traits, can prevent the evolution of between-genotype cooperation. *Upper* illustrates temporal dynamics (in generations G) for the three outcomes of the model. The cost per secretion (c) was set at 5%.

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Supporting Information

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SI Materials and Methods

Here, we present an extended version of the methods used in the paper.

Ordinary Differential Equation Models with Fixed Investment into Secretion. There is a large literature on the evolution of single microbial secretions—often known as studies of cheaters and cooperators—that includes both theory (1–4) and empirical tests (5–8). Our goal here is to build directly on this work. We, therefore, base our models on two recent and detailed studies of single secretions (3, 7). These studies assume that cells land in patches stochastically, such that the number of cells across patches follows a Poisson distribution (Poisson-based seeding) (Fig. S9). Such seeding is based on the realistic assumption that the number of cells per patch is not deterministic and the same across patches but random. Earlier models used simpler demographics, but this difference does not affect our conclusions (Fig. S9), such that our model also recapitulates the predictions of other studies of single secretions (2, 4).

We model the population dynamics of microbial genotypes growing in a well-mixed environment using systems of ordinary differential equations (ODEs). Each system is composed by 2^s equations, where s is the number of traits (secretions) in a given model, and each equation represents the population dynamics of a given microbial genotype in each subpopulation. The total number of genotypes in each model is, thus, captured by binary combinatorics; each genotype is labeled by a binary string, where one and zero mean production or nonproduction of a certain trait, respectively. Here, we explain the two-trait model, but the framework is equivalent for the one- and three-trait cases presented in the text. For a system of two cooperative traits, the four genotypes ([1,1], [1,0], [0,1], and [0,0]) grow according to the following general form:

$$\frac{dg_{ij}}{dt} = (r - (i+j) \cdot c) \cdot g_{ij} \cdot \left(1 - \frac{\sum g}{K}\right),$$

where g_{ij} represents the density of individuals of genotype $[i,j]$ in each subpopulation $[(i,j) \in \{1,0\}^s]$, and s is the number of traits. Specifically for the two-trait model, one has the following system of four ODEs:

$$\begin{cases} \frac{dg_{11}}{dt} = (r - (1+1) \cdot c) \cdot g_{11} \cdot \left(1 - \frac{g_{11} + g_{10} + g_{01} + g_{00}}{K}\right) \\ \frac{dg_{10}}{dt} = (r - (1+0) \cdot c) \cdot g_{10} \cdot \left(1 - \frac{g_{11} + g_{10} + g_{01} + g_{00}}{K}\right) \\ \frac{dg_{01}}{dt} = (r - (0+1) \cdot c) \cdot g_{01} \cdot \left(1 - \frac{g_{11} + g_{10} + g_{01} + g_{00}}{K}\right) \\ \frac{dg_{00}}{dt} = (r - (0+0) \cdot c) \cdot g_{00} \cdot \left(1 - \frac{g_{11} + g_{10} + g_{01} + g_{00}}{K}\right) \end{cases},$$

where r is the intrinsic growth rate (here assumed to be $r = 1$ for simplicity), and c is the cost of producing each trait. Note that we assume here additive costs for secretion production (given by the summation of the genotype index). We also considered nonlinear costs (both accelerating and decelerating), and these functional forms do not qualitatively affect our conclusions (Fig. S6). Importantly, K (the carrying capacity of the environment) is

a function of the traits available (here given by the frequency of producers of each trait) and has the following general form:

$$K = K_{basal} + K_{maxB} \left(\frac{g_{11} + g_{10}}{g_{11} + g_{10} + g_{01} + g_{00}} \cdot \frac{g_{11} + g_{01}}{g_{11} + g_{10} + g_{01} + g_{00}} \right),$$

where K_{basal} and K_{maxB} are constants representing the minimal carrying capacity of the system and the maximum benefit from secretions, respectively (here, $K_{basal} = 1$ and $K_{maxB} = 100,000$). Note that we use multiplicative benefits, because we assume that all cooperative traits are essential, which together with $K_{basal} = 1$, represents the requirement of cells having access to all secretions to be able to grow. However, we also vary the strength of selection by considering increasing values of K_{basal} . Fig. S1 shows that increasing K_{basal} reduces the potential for between-genotype cooperation, but when it evolves, it does so under the same demographic conditions and intermediate values of genetic mixing. Moreover, given that it is not fully known how K scales with secretions, we additionally implemented an additive version, where now we consider additive benefits instead of multiplicative benefits (Fig. S8). Again, the evolution of between-genotype cooperation is restricted to intermediate values of genetic mixing. To model the effect of niche overlap on the evolution of between-genotype cooperation, we analyzed an extension of the two-trait model presented above, where we considered eight genotypes belonging to two different species. In this case, only genotypes from the same species compete for resources (Fig. S4).

We consider a standard lifecycle of microbes [the work by Cremer et al. (3) and references therein further discuss the lifecycle]: (i) formation, where a random number of cells is allocated to each subpopulation (based on a Poisson process with mean n_0) in a set of M subpopulations (here, $M = 1,000$) and the identity of each cell follows a uniform distribution based on the frequency of each genotype [which we assumed is the same for all genotypes at the beginning ($G0$)]; (ii) growth, where genotypes in each group proliferate according to logistic dynamics (until saturation is reached); and (iii) merging, where the M groups are merged together and the global genotypic frequencies are updated. After a full cycle of seeding, growth, and merging (equivalent to one generation), new patches are seeded according to the recalculated genotypic frequency. These cycles are repeated for G generations (typically 1,000), which is sufficient for genotypic frequencies reaching equilibrium.

ODE Models with Variable Investment into Secretion. To model the evolution of investment into secretion production, we implemented two different frameworks (here called discrete and continuous models for the evolution of investment). In the discrete version, we simply extended our model described above to allow strains to invest in three different levels of secretion (0.5, 1, and 2). We, thus, considered the genotypes [0.5,0.5], [1,1], [2,2], [0.5,0], [1,0], [2,0], [0,0.5], [0,1], [0,2], and [0,0] and show that [2,2] evolves under low mixing but that [0.5,0] and [0,0.5] will evolve under the conditions favoring cross-feeding. Here, we assume for simplicity that full producers invest the same to both secretions, but we find the same qualitative results when we drop this assumption (Fig. S5).

In the continuous version, we use the same lifecycle but add a mutation phase, where we allow for mutations that can either increase or decrease the investment into cooperation. The lifecycle is then (i) formation; (ii) mutation, where each cell of the subpopulation mutates its parameter x , which governs how much

a cell invests into its public goods; (iii) growth; and (iv) merging. In the growth phase, the evolution of each single subpopulation is governed by a set of z ODEs, where z is the number of individuals that inhabit each subpopulation after mutation. Each initial cell z then proliferates according to the general ODE

$$\frac{dg_{x,ij}}{dt} = (r - (i+j) \cdot x \cdot c) \cdot g_{x,ij} \cdot \left(1 - \frac{\sum g}{K}\right),$$

where $g_{x,ij}$ represents the density of the cell type with genotype $[i,j]$ and investment x , and K is still a function of the secretions available (given by the frequency of secretors), which now incorporates explicitly the evolvable investment x of cells:

$$K = K_{\text{basal}} + K_{\text{maxB}} \left(\frac{x \cdot g_{11} + x \cdot g_{10}}{g_{11} + g_{10} + g_{01} + g_{00}} \cdot \frac{x \cdot g_{11} + x \cdot g_{01}}{g_{11} + g_{10} + g_{01} + g_{00}} \right).$$

Thus, the genotypes are now effectively $x \cdot g_{ij}$ and not g_{ij} as before. For example, if $x = 2$, $[1,1]$ will behave as a genotype $[2,2]$, because we assume for simplicity that full producers will evolve the same level of investment for both traits.

Individual-Based Model. We used an individual-based model to examine the effects of spatial structure on our predictions. In particular, we ask if between-genotype cooperation can evolve if secreted traits have limited diffusion, which may be common on surfaces (9). To capture the effect of diffusion, we use spatial simulations that consider 2D lattices with periodic boundaries. Cells can only divide into empty spaces of their closest neighborhood (eight spaces around the focal individual), and the effect of a public good is limited to a neighborhood of a certain size around the producer cell. This spatial version is based on the same assumptions as the previous model for homogenous environments, and it uses an adaptation of the Gillespie algorithm for simulating population dynamics in continuous time (10). Although exactly equivalent to the Direct Method by Gillespie (11), this alternative is computationally more efficient and can easily handle large populations of individuals with individual properties

according to rules for cellular growth. At each sampled time step, a focal individual is selected with uniform probability, and a cell division event is then realized with a probability directly proportional to its growth rate, which depends on population density and the presence of public goods following logistic kinetics. The stochasticity of cell division adds extra variance to the final genotypic composition of the simulated patches compared with the deterministic growth in the ODE version. Thus, the results of the individual-based model and the ODE model are not always identical for identical parameter values, with cooperation typically emerging more easily in the individual-based model. Nevertheless, we can use the individual-based model to specifically evaluate the effects of spatial structure by comparing a high-diffusion case, where between-genotype cooperation emerges, with a low-diffusion case (here given by a neighborhood of 24 cells from the source).

We implemented the standard three-step lifecycle like for the deterministic version. First, a number of M separate lattices is seeded according to the initial genotypic frequencies. Second, each subgroup develops separately, with cell growth being spatially simulated. Third, the subgroups are merged to update the genotypic frequencies, which are used for the following seeding step. Importantly, we let the yield of a focal subgroup depend on the presence of traits (secretions) in this group, with the consequence that subgroups with more producers will have higher final yield. Although in the ODE model K changed continuously with the number of producers, in the individual-based model K remained constant for the duration of the growth phase, but then, we weighted subgroups according to the proportion of producers present at the end of the simulation before the merging step. This implementation captures the dependence of subgroup yield on the proportion of producers in a group in a manageable way, but it differs from the ODE model. This different implementation of secretion-dependent carrying capacity could potentially affect comparisons between the two model frameworks. However, as discussed above, we only directly compare the cases of global and local diffusion within the individual-based model framework.

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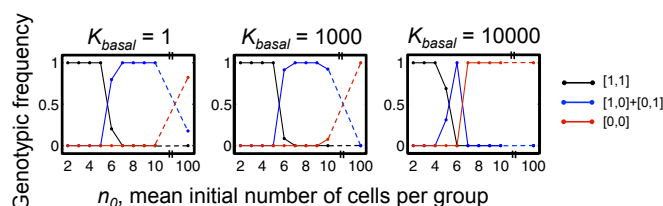


Fig. S1. Effect of basal fitness on the evolution of between-genotype cooperation in microbes. Our main models assume that secreted traits are essential for growth, which means that groups without secretions will not grow. Here, we vary K_{basal} (carrying capacity without secretions) from 1 to 10,000 [up to 10% of the maximum benefit from secretions (K_{maxB})] (SI Materials and Methods). The only effect is to reduce the scope for between-genotype cooperation, which further suggests that cooperation among microbial genotypes is limited. Results from the two-trait model (ODE version) with the cost (c) at 5% of growth rate are shown.

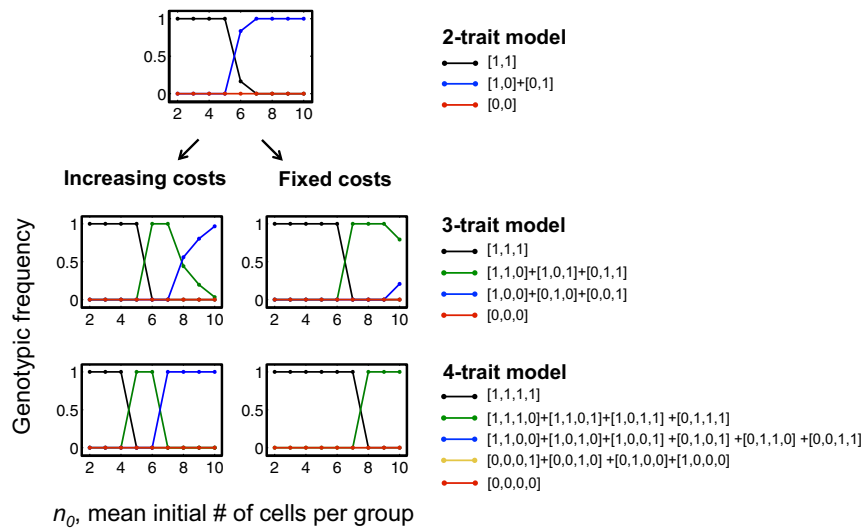


Fig. S2. Effect of increasing the number of traits on the evolution of cross-feeding. (*Lower Left*) Systems that rely on four secretions select more for cooperative cross-feeding than those relying on three secretions only. This results from the increasing cost of adding another secretion to a genotype that produces all traits. (*Lower Right*) If total costs are fixed (here, at 10% of maximum growth rate), then adding another secretion reduces the prevalence of cross-feeding.

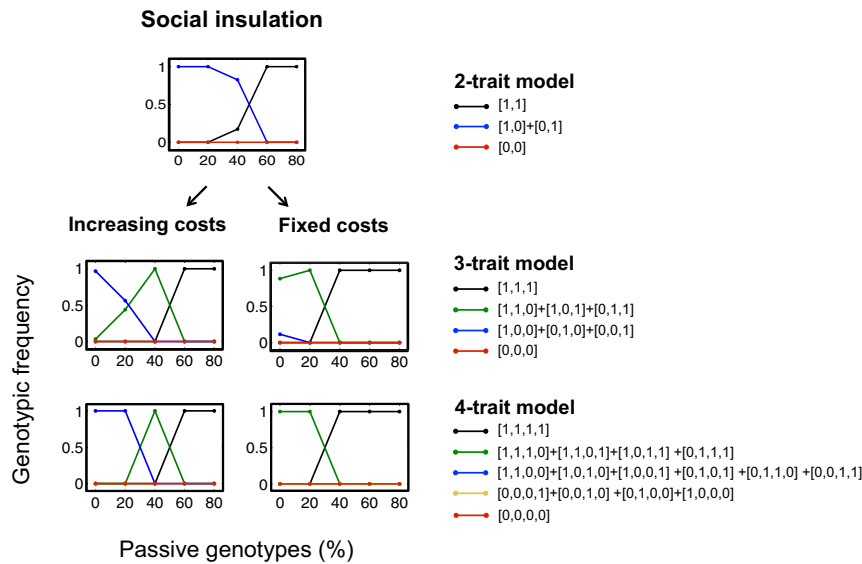


Fig. S3. Social insulation in systems with increasing numbers of traits. The introduction of passive genotypes has the same effect on the evolution of cooperative cross-feeding in systems relying on two, three, and four traits: a high percentage of passive genotypes prevents between-genotype cooperation. (*Lower Right*) Holding the total cost of all secretions constant shows that social insulation is particularly problematic for systems with many secretions. Here, we fixed total costs at 10% of maximum growth rate and genetic mixing at $n_0 = 10$, which promote cross-feeding in the absence of passive genotypes.

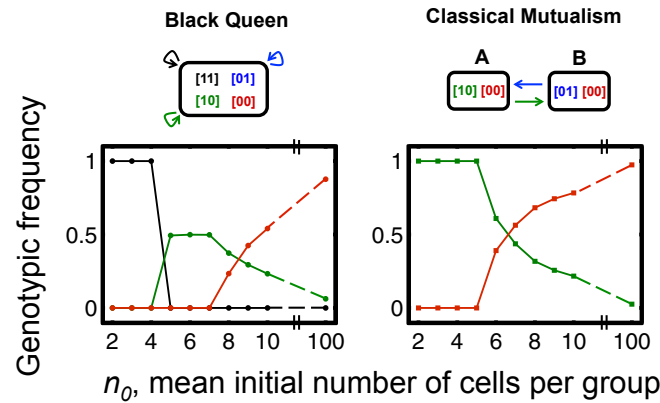


Fig. S7. Evolution of the genotypic cooperation in our model (Black Queen-like) compared with a classical mutualism model. We modeled classical mutualism by considering two species of bacteria (species A and B) that do not compete for the same resources and possess unique traits (one secretion each) that can only be used by the partner species for growth. That is, there is no [1,1] genotype but just a single-trait producer and respective cheater genotype within each species. For both cases, we considered well-mixed conditions and high secretion cost ($c = 10\%$ of maximum growth rate per secretion). The potential for genotypic cooperation is higher under canonical mutualistic interactions (here given by a wider region of genotypic mixing, under which cooperative partners dominate the population). The within-species frequencies of [1,0] and [0,1] in both plots overlap, as expected, and therefore, only one of two colors is seen in the plot.

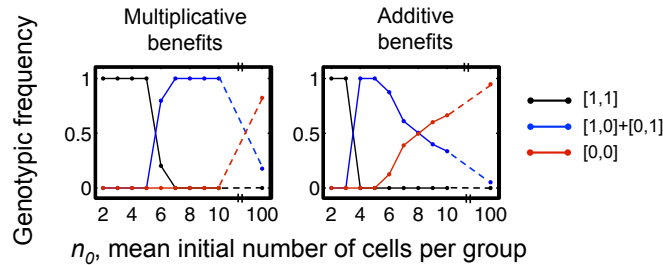


Fig. S8. Effect of the functional form of benefits on the evolution of between-genotype cooperation in microbial communities. Qualitative conclusions are robust to both multiplicative and additive benefits to secretions. Our main models assume that the benefits of multiple secretions combine multiplicatively to set population carrying capacity (K). For example, for two traits, K is proportional to the product of the amount of secretions 1 and 2 in each subpopulation. Here, this scenario is compared with a case where K scales as the sum of the amounts of two secretions. Results are shown from the two-trait model (ODE version) with cost (c) at 5% of the maximum growth rate, where the two fitness functions are, for multiplicative benefits,

$$K = K_{\text{basal}} + K_{\text{maxB}} \left(\frac{g_{11} + g_{10}}{g_{11} + g_{10} + g_{01} + g_{00}} \cdot \frac{g_{11} + g_{01}}{g_{11} + g_{10} + g_{01} + g_{00}} \right),$$

and for additive benefits,

$$K = K_{\text{basal}} + K_{\text{maxB}} \left(\frac{g_{11} + g_{10}}{g_{11} + g_{10} + g_{01} + g_{00}} + \frac{g_{11} + g_{01}}{g_{11} + g_{10} + g_{01} + g_{00}} \right) / 2.$$

As before, K_{basal} and K_{maxB} are the minimal carrying capacity of the system and the maximum benefit from secretions, respectively, and g_{ij} is the density of individuals of genotype $[i,j]$ in each subpopulation.

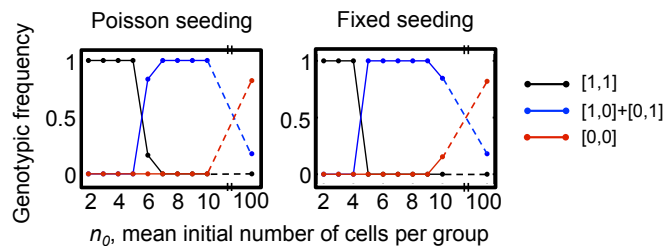


Fig. S9. Effect of the type of seeding on the evolution of cooperative cross-feeding. We find the same qualitative behavior for (Left) Poisson-based seeding, where a random number of cells is initially introduced in each group, and (Right) fixed seeding, where we introduce the same number of cells in all groups. However, there are some quantitative differences, because fixing the number of cells introduced per group reduces the variance between groups. This reduction in variance between groups affects the potential for Simpson's Paradox and thus, the evolution of cooperation both within and between genotypes. Costs per trait were set at 5% of the maximum growth rate.