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Synergy and Group Size in Microbial Cooperation

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Online enhancement: appendix.

ABSTRACT: Microbes produce many molecules that are important for their growth and development, and the exploitation of these secretions by nonproducers has recently become an important paradigm in microbial social evolution. Although the production of these public-goods molecules has been studied intensely, little is known of how the benefits accrued and the costs incurred depend on the quantity of public-goods molecules produced. We focus here on the relationship between the shape of the benefit curve and cellular density, using a model assuming three types of benefit functions: diminishing, accelerating, and sigmoidal (accelerating and then diminishing). We classify the latter two as being synergistic and argue that sigmoidal curves are common in microbial systems. Synergistic benefit curves interact with group sizes to give very different expected evolutionary dynamics. In particular, we show that whether and to what extent microbes evolve to produce public goods depends strongly on group size. We show that synergy can create an “evolutionary trap” that can stymie the establishment and maintenance of cooperation. By allowing density-dependent regulation of production (quorum sensing), we show how this trap may be avoided. We discuss the implications of our results on experimental design.

Keywords: synergy, microbes, cooperation, public goods, quorum sensing.

Introduction

Cooperative behavior is a ubiquitous feature of interactions at all levels of biology, and a large body of theory has been developed to explain the apparent paradox of its evolution and maintenance in the face of noncooperative social cheats (e.g., Smith and Szathmáry 1995; Keller 1999; West et al. 2006). Over the past decade, the use of microbes has proven effective in testing this theoretical work. Microbes reproduce very quickly and are relatively easy to control and genetically manipulate. They have been used to elucidate major parts of social-evolution theory, such

as the evolution of altruism and spite, and to support related evolutionary hypotheses (Gardner et al. 2004; Griffin et al. 2004; West et al. 2006). Additionally, microorganisms are fascinating in their own right, and an understanding of their sociality can have major health consequences (Brown et al. 2009).

In microbes, among the most studied social traits is the production of secreted compounds, for example, those that scavenge for iron when iron is limited (Griffin et al. 2004) or those that convert sucrose to glucose when glucose is limited (MacLean and Gudelj 2006; Gore et al. 2009). Investigations in the past decade have only begun to explore the abundance and diversity of potential public-goods secretions in nature (West et al. 2006). Most of this work has focused on Hamilton’s rule ($rb > c$) as the condition that favors the evolution of social traits. A primary focus has been on population structuring (shaping Hamilton’s r), although all three quantities are essential in social evolution (West et al. 2006).

In this article, we focus primarily on fitness effects (shaping Hamilton’s b and c)—specifically, the group-beneficial traits of public-goods production. Figure 1A shows three plausible benefit functions: one decelerating, one accelerating, and one sigmoidal (first accelerating and then decelerating). We use the term “synergy” to refer to acceleration in benefit functions, represented by the first part of the sigmoidal function and all of the accelerating function in figure 1A. This synergy allows per capita fitness benefits to increase with group size, as shown in figure 1B; this is sometimes termed “group augmentation” (Kokko et al. 2000). Synergy is abundant in biology, from ant pheromone trails to human architecture (Clutton-Brock 2002; Sumpter and Brännström 2008; Sumpter 2010). The mechanisms that cause this synergy vary widely (Sumpter and Brännström 2008). Despite the abundance of synergy in other cooperative organisms, the phenomenon is not well investigated in microbes. Although recent work has uncovered benefit nonlinearities in *Myxococcus* and in an engineered *Escherichia coli* system (Chuang et

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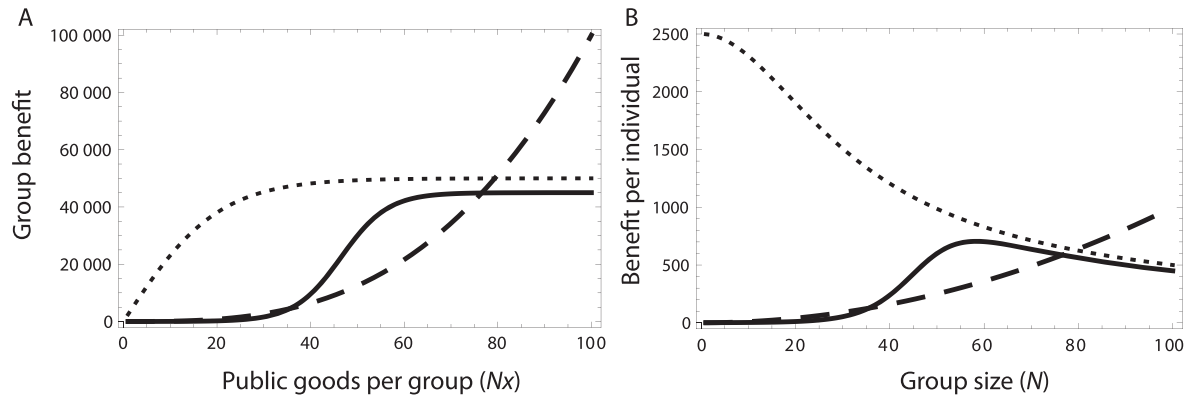


Figure 1: Accelerating, decelerating, and sigmoidal benefit functions. A, Total benefit $B(Nx)$ as a function of total public-goods investment Nx , expressed as the product of the group size N and the average cooperative investment per individual x . The solid and dashed lines represent synergistic (accelerating) benefits, as they have positive concavity for some intervals, whereas the dotted line always has diminishing returns. B, Corresponding per capita benefit $B(Nx)/N$ as a function of group size N , assuming that every individual cooperates at some fixed amount (here $x = 1$, indicating full cooperation). The benefit functions used are $B(x) = \alpha[\beta + d \exp(\kappa - bx)]^{-1} - \alpha[\beta + d \exp(\kappa)]^{-1}$ with $\alpha = 10^5$, $d = 1$, $\beta = 1$, $\kappa = 0$, and $b = 0.1$ (decelerating benefits; dotted line); $B(x) = bx^\alpha$, where $b = 0.1$ and $\alpha = 3$ (accelerating benefits; dashed line); and $\alpha[\beta + d \exp(\kappa - bx)]^{-1} - \alpha[\beta + d \exp(\kappa)]^{-1}$, where $\alpha = 90,000$, $d = 1$, $\beta = 2$, $\kappa = 10$, and $b = 0.2$ (sigmoidal benefits; solid line).

al. 2010; Smith et al. 2010), it is generally ruled out in mathematical models (West and Buckling 2003; Foster 2004; Ross-Gillespie et al. 2009), and the scope and impact of these nonlinearities have not been well investigated. Here we argue that although its sources and abundance are still not well understood, synergy is likely to be a key factor in microbe evolution.

Synergy occurs any time each additional public-goods molecule that is produced gives a greater fitness benefit than the previous one. Many biological molecules can provide benefits in a sigmoidal fashion due to “positively cooperative reactions” (Hill 1910). Aside from basic molecular properties, sigmoidal benefits are often expected from ecological interactions. For instance, the normal life cycles of many invading parasites require production of threshold quantities of toxins to function and/or be able to overwhelm the immune system (Williams et al. 2000). Synergy may also arise from less apparent and more complex ecological interactions. For example, an adversarial molecule that up to some point neutralizes produced public goods could lead to synergy, such as plants that produce “quorum-quenching” molecules, which neutralize quorum-sensing molecules (the production of which is, itself, cooperative; Diggle et al. 2007). Synergy could even be caused by the consumption of others’ public goods, as in, for example, the siderophore pyoverdine secreted by *Pseudomonas aeruginosa* (Brown and Kümmerli 2010).

Most theoretical studies of cooperative microbial interactions typically preclude synergy (Brown and Johnstone 2001; West and Buckling 2003; Foster 2004; Ross-Gillespie

et al. 2009). It has previously been concluded that high density is disfavorable for public-goods cooperation, because a producing cell enjoys a growth benefit proportional to the average production of its neighbors, which allows nonproducers more opportunities to exploit high-producing cells (Ross-Gillespie et al. 2009). As we shall see here, the opposite conclusion is possible when synergy exists. Synergy has been previously studied theoretically, in other contexts where an individual is classified as either a cooperator or defector (Queller 1985; Archetti 2009a, 2009b), and more recently in a continuous-trait model (Deng and Chu 2011; see Archetti 2009b for a review on nonlinear public goods games in biology). Here we extend these studies to continuously variable traits to understand aspects of evolution not examined in previous frameworks. We show that synergy often permits the existence of an evolutionary repeller near nonproduction, which can have important consequences, and we demonstrate the role that quorum sensing may play in its avoidance.

If the fitness effects of public-goods production depend on density, then this dependence should provide evolutionary pressure for microbes to regulate their production by density. By releasing autoinducer molecules, a cell can assess how many other cells surround it, a proxy for cellular density, and it can up- or downregulate the expression of various genes in response (Fuqua et al. 1996; Robson et al. 1997). This mechanism, known as quorum sensing, regulates the expression of many genes for the secretion of extracellular molecules. In this article, we also inves-

tigate the coevolution of quorum sensing and a synergistic trait.

Model

We adopt a standard setting for studying games with continuous cooperative investments (Doebeli et al. 2004). We consider an infinite population in which each individual i has a strategy or trait value x_i , which represents the individual's level of cooperative investment. For simplicity's sake, we will assume that individual cooperative investments are constrained between 0 and 1, that is, $0 \leq x_i \leq 1$. The demographic dynamics unfold in discrete generations. At each generation, groups of size N are randomly formed. Assuming that the players in one of these groups have the respective strategies x_1, \dots, x_N , the payoff for an individual i in the group where $1 \leq i \leq N$ is given by

$$\frac{B(x_1 + \dots + x_N)}{N} - C(x_i). \quad (1)$$

Here, $B(x_1 + \dots + x_N)$ is the collective benefit of the group's cooperative investment (fig. 1A). The collective benefit is divided equally among the N individuals in the group to give the per capita benefit (fig. 1B), and $C(x_i)$ is the cost of the investment. Both the benefit and cost functions are assumed to be strictly increasing.

The selection gradient,

$$D(x) = \frac{B'(Nx)}{N} - C'(x), \quad (2)$$

indicates the direction of gradual evolutionary change in a monomorphic population of individuals that all have the same trait value x ; primes denote first derivatives. For details on how the selection gradient is derived from the demographic model and how it relates to gradual evolutionary change, see articles by Geritz et al. (1997), Sumpter and Brännström (2008), Doebeli et al. (2004), and Dieckmann and Law (1996). With small mutational steps, the evolutionary dynamics will proceed in the direction indicated by the selection gradient and will cease only at a boundary strategy or at an interior strategy x^* , at which point the selection gradient vanishes, $D(x^*) = 0$. Such strategies are said to be evolutionarily singular. If nearby monomorphic populations evolve toward the singular strategy, the strategy is said to be convergence stable. A convergence-stable singular strategy can be either evolutionarily stable (then called continuously stable)—effectively, an endpoint of evolution—or an evolutionary branching point, at which a monomorphic population may gradually diverge to become dimorphic (Geritz et al. 1997). Mathematically, the requirement that a singular

strategy is convergence stable but not evolutionarily stable can be written as follows:

$$B''(Nx^*) < C''(x^*) < \frac{B'(Nx^*)}{N} < 0, \quad (3)$$

where double primes denote second derivatives. We refer to articles by Doebeli et al. (2004) and Geritz et al. (1997) for details. In words, this condition implies that both the benefit function B and the cost function C must be decelerating in the vicinity of the singular strategy x^* .

Group size has been considered previously as the number of founders initiating a microbe colony (see Brännström and Dieckmann 2005; Brännström et al. 2010), but our analysis takes N to be the size of the group of interacting cells or neighboring bacteria within a public-goods molecule's radius of diffusion; this definition is often a proxy for cellular density. To consider the impacts of genetic assortment, we assumed that when a focal individual joins a group at the beginning of a generation, some positive fraction of the other individuals in the group may be identical to it by descent. This fraction is a random variable ρ . We show in the appendix, available online, that with these assumptions, the selection gradient takes the form

$$D(x) = \frac{B'(Nx)[1 + \langle \rho \rangle (N - 1)]}{N} - C'(x), \quad (4)$$

where angle brackets indicate the expectation. The condition for convergence stability is

$$B''(Nx^*)[1 + \langle \rho \rangle (N - 1)] - C''(x^*) < 0, \quad (5)$$

and the criterion for evolutionary stability is

$$\frac{B''(Nx^*)}{N} [(1 + (N - 1)\langle \rho \rangle)^2 + \text{Var}(\rho)(N - 1)^2] < C''(x^*). \quad (6)$$

Again, both the benefit function B and the cost function C must be decelerating in the vicinity of the singular strategy x^* for evolutionary branching to occur. Interestingly, we see that the mean of ρ (which is simply others-only relatedness; see the appendix) is sufficient to define convergence stability, but both the mean and the variance of ρ are required to define evolutionary stability. Higher variance in ρ makes conditions for evolutionary branching more restrictive.

We also consider the effects of group size-dependent regulation (quorum sensing) on the maintenance of cooperation. Here an individual can alter its behavior, depending on the size of its group. At sufficiently low group sizes, the public goods are not produced, or they are produced in very little quantity, whereas at sufficiently high group sizes, production is simply the productive invest-

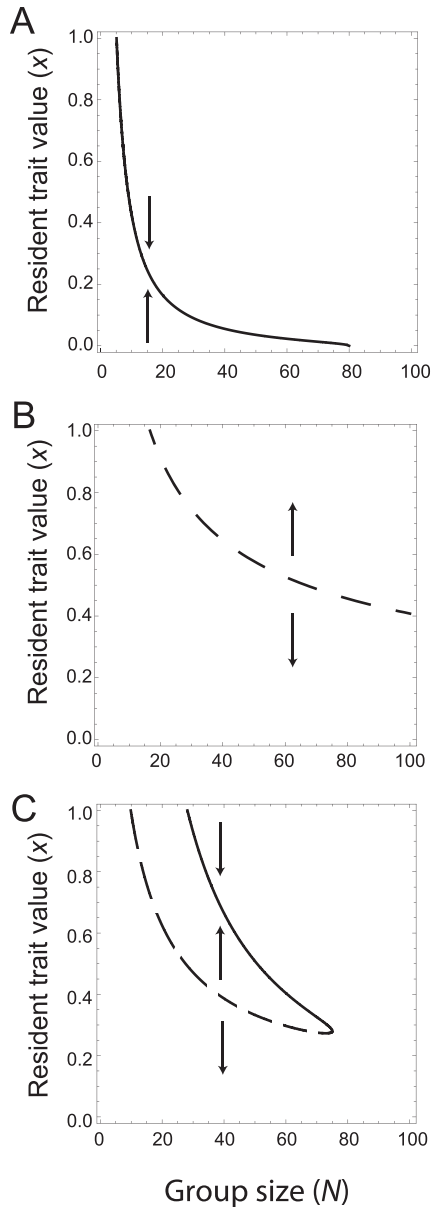


Figure 2: Bifurcation plots illustrating the evolutionary dynamics with decelerating, accelerating, and sigmoidal benefit functions. The solid lines indicate the location of interior singular strategies for different group size N . The arrows indicate the direction of gradual evolution of a monomorphic population. *A*, Decelerating benefits. There is a unique evolutionarily stable strategy that decreases with group size. When group sizes are greater than ~ 80 , cooperation is entirely disfavored. $B(x) = \alpha[\beta + d \exp(\kappa - bx)]^{-1} - \alpha[\beta + d \exp(\kappa)]^{-1}$, where $\alpha = 2,000$, $d = 1$, $\beta = 1$, $\kappa = 0$, and $b = 0.8$. *B*, Accelerating benefits. For small group sizes there is a unique evolutionarily stable strategy corresponding to full defection. As group size increases, full cooperation also becomes an evolutionarily stable strategy. Any interior singular strategy is repelling and decreases with group size but never reaches zero. $B(x) = bx^\alpha$, where $b = 0.1$ and $\alpha = 3$. *C*, Sigmoidal benefits. Up to two interior singular strategies are possible, one repelling and the other attracting. $B(x) = \alpha[\beta + d \exp(\kappa - bx)]^{-1} - \alpha[\beta + d(\kappa)]^{-1}$, where $\alpha = 10,000$, $d = 1$, $\beta = 2$, $\kappa = 7$, and $b = 0.3$. Each convergent stable attractor could potentially be an evolutionary branching point. The cost function used is $C(x) = cx$, where $c = 5$.

ment x . The payoff to individual i with trait values (x_i, s_i) in this model variant is simply

$$\frac{B(Q(s_i, N)x_i + \dots + Q(s_N, N)x_N)}{N} - C(Q(s_i, N)x_i), \quad (7)$$

where $Q(s_i, N) = 1/[1 + \exp(s_i - N)]$. Here s_i approximates the group-size threshold above which an individual i produces public goods and below which it does not (see the appendix for further details).

Results

We begin by assuming that groups are formed entirely randomly within the entire population, so that there is no correlation among phenotypes of group members. Figure 2A shows the evolutionary dynamics under small mutational steps when the benefit is a decelerating function of the group's total cooperative investment (dotted line in fig. 1; for diminishing returns, see also Foster 2004). Here, per capita benefits decrease with group size. Assuming that the cost is proportional to the investment $C(x) = cx$, it follows that the selection gradient $D(x) = B'(Nx)/N - c$ is strictly decreasing with x , so there is exactly one convergence-stable singular strategy that can be shown to also be evolutionarily stable, hence a continuously stable strategy (CSS; both convergence stable and evolutionarily stable) that is either full defection, an intermediate level of cooperation, or full cooperation (fig. 2A). This CSS, once reached, is resistant to invasions by mutants sufficiently phenotypically close on either side of it. This is very much the standard picture of evolutionary games involving costs and benefits to cooperation; the evolutionarily stable cooperative investment decreases with group size N (Ross-Gillespie et al. 2009).

Whereas figure 2A illustrates the implications of the most common assumptions about benefit functions, it is not necessarily the case that increasing total producers leads to decreasing per capita benefits. The alternative scenario is the one in which the per capita benefit derived from cooperative interactions increases with group investment (fig. 2B). Here, for linear cost functions, the selection gradient $D(x)$ is strictly increasing and there is

lutionarily stable strategy corresponding to full defection. As group size increases, full cooperation also becomes an evolutionarily stable strategy. Any interior singular strategy is repelling and decreases with group size but never reaches zero. $B(x) = bx^\alpha$, where $b = 0.1$ and $\alpha = 3$. *C*, Sigmoidal benefits. Up to two interior singular strategies are possible, one repelling and the other attracting. $B(x) = \alpha[\beta + d \exp(\kappa - bx)]^{-1} - \alpha[\beta + d(\kappa)]^{-1}$, where $\alpha = 10,000$, $d = 1$, $\beta = 2$, $\kappa = 7$, and $b = 0.3$. Each convergent stable attractor could potentially be an evolutionary branching point. The cost function used is $C(x) = cx$, where $c = 5$.

either one or two CSSs corresponding to no cooperative investment or zero full cooperative investment separated by a repeller. Typically, for small groups, no investment is the only evolutionarily stable strategy, but at some critical group size full investment also becomes continuously stable (fig. 2B).

In a biologically realistic setting, synergistic interactions cannot continue indefinitely, and there is a critical level at which the per capita benefit of additional cooperative investment begins to decrease. This leads to a sigmoidal form of the benefit function B . In this situation, the evolutionary dynamics can incorporate elements of both cases discussed above. This can be seen in figure 2C, where there are two branches of singular strategies: the lower branch is repelling (because it comes from the synergistic lower part of the benefit function), and the upper branch is attracting (because of the decelerating portion of the benefit function that occurs at higher cooperative investments). In this example, cooperation first becomes possible at intermediate sizes and then suddenly shifts to no cooperation at high group sizes.

Thus far, we have assumed that costs are proportional to the amount invested, leading to a linear cost function. When costs are nonlinear, the population can sometimes become dimorphic in the vicinity of a singular strategy. When this occurs, the two evolutionary branches diverge and may eventually end up on opposite sides of the CSS, in a process known as evolutionary branching (Geritz et al. 1997). In this case, the branches may also end up on opposite sides of the evolutionary repeller caused by the synergistic part of the benefit curve. Figure 3A–3C demonstrates (assuming no assortment) that not only is coexistence possible when the benefits are synergistic, but the coexisting community can emerge from an initially monomorphic community in small evolutionary steps. Figure 3B and 3C shows the evolutionary dynamics when group size is fixed ($N = 30$). If the population starts with a trait value of less than $x \approx 0.2$, evolution proceeds toward zero investment. If, instead, the trait value is initially greater than this threshold, evolutionary branching occurs and a stable coexistence between full producers and nonproducers is eventually achieved.

Figure 3D, which depicts population dynamics between two strategies, shows that coexistence is maintained by frequency-dependent population dynamics. If, however, a high enough frequency of nonproducers accumulates for any reason in the population, the producers will be eliminated, leaving only nonproducers. Figure 3 shows a wide range of outcomes for a single benefit-and-cost function: full investment by all, full investment by some proportion of the population and zero investment by all others, and zero investment by all, dependent only on initial conditions and group size. In this example, all singular strategies

are evolutionarily unstable, but as equation (3) shows, group size can also determine whether a population evolves toward an intermediate evolutionarily stable investment level or whether a dimorphic population emerges.

We have until now assumed random assortment among all individuals in the population ($\rho = 0$). This may be acceptable in situations where the individuals are interpreted as founders that may have dispersed from afar, but in many other biological contexts the groups will not be well mixed. As shown in figure 4, high assortment makes cooperation more likely to evolve; compared with plots in figure 2 in the bifurcation plots of singular strategies, the repelling branches (convergence unstable) are lowered and the attracting (convergence stable) branches are raised. This is because the benefits grow but there is no longer the need to split the spoils among many genotypes. This relates to previous work with discrete types that has demonstrated that assortment decreases the critical cost for a mixed equilibrium between cooperators and defectors (Archetti 2009a, 2009b). Assortment is especially important in large groups where direct benefits alone are not sufficient to maintain cooperation. Sufficiently high relatedness values may allow cooperation to be maintained at some positive level for arbitrarily high group sizes, even when the benefit function is diminishing or sigmoidal (in contrast to fig. 2). If cooperation is convergence stable at some group size, with no assortment ($r_0 = 0$), then in a clonal population ($r_0 = 1$), a positive level of cooperation is also convergence stable at arbitrarily high group sizes (see appendix). Equations (5) and (6) make clear that the conditions for evolutionary branching are dependent on both the mean and the variance of ρ . We give an example in the appendix where changing $\text{Var}(\rho)$ can alter evolutionary stability without affecting convergence stability, thus determining whether evolutionary branching occurs.

In figure 5, we show the joint evolution of the trait for public-goods production and the quorum-sensing threshold group size above which the trait is expressed (see eq. [7]). In this case, the costs and benefits of expression correspond to those in figure 2C. At each generation, a group is formed of size 5 or size 45 with equal probability. When the public good is constitutively produced, the population always ends in nonproduction and cannot recover, even at large group sizes. In contrast, when group size-dependent regulation of the trait is allowed to evolve, the cooperation can be maintained. The threshold (s) can evolve such that the cooperative trait is not expressed at the low group size (5) but is expressed at other group sizes when it is advantageous (see appendix for an example). Figure 5 also reveals that when the investment value x is sufficiently low, the threshold s is expected to increase, which can make investment subsequently less likely to increase.

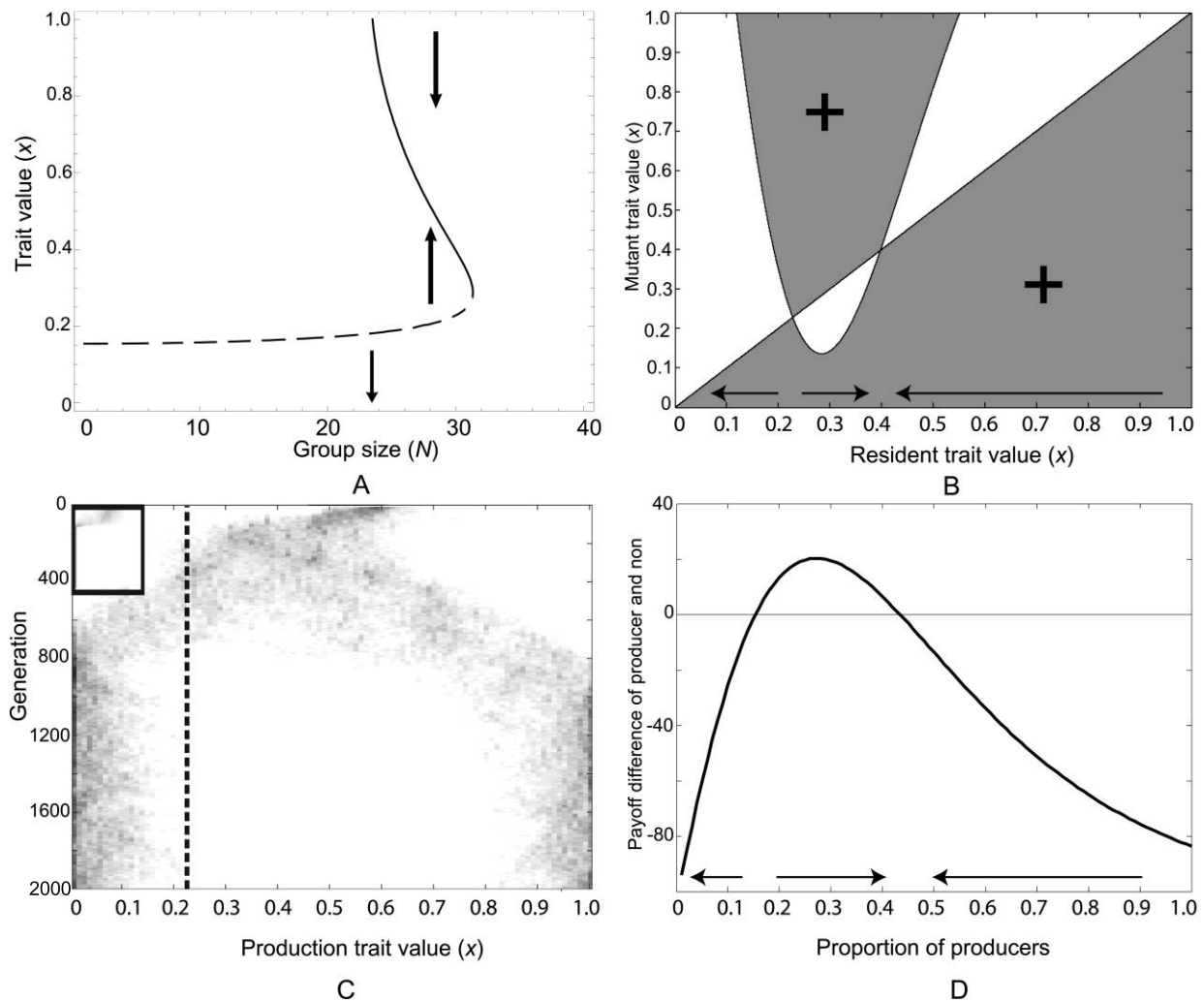


Figure 3: Evolutionary branching and the emergence of two coexisting strategies of full defection and full cooperation in a setting with sigmoidal benefits and nonlinear costs. *A*, Bifurcation plot illustrating the effects of group size on directional selection for the function used in the other diagrams in the figure. *B*, Pairwise invasibility plot (PIP) illustrating the monomorphic evolutionary dynamics. There are singular strategies at approximately $x = 0.2$ and $x = 0.4$, of which only the latter is convergence stable. Monomorphic populations with trait values above the first singular strategy will evolve toward the second singular strategy, where they undergo disruptive selection and, subsequently, evolutionary branching. *C*, Individual-based simulation demonstrating evolutionary branching at approximately $x = 0.4$, thus corroborating the predictions from the PIP. The inset shows the evolutionary dynamics for populations that initially have trait values lower than the first singular strategy at approximately $x = 0.2$; here investment decreases to zero. *D*, Population dynamics of the resultant coexisting strategies of full defection ($x = 0$) and full cooperation ($x = 1$). If the fraction of cooperators is initially less than $\sim 18\%$, the cooperators will be eliminated altogether. Otherwise, the population dynamics will result in a stable coexistence with $\sim 45\%$ cooperators. The sigmoidal benefit function used is the same as in figure 1, $B(x) = b(x^3 + \beta x^2)(x^2 + \alpha)^{-1}$, but with different parameters: $b = 200$, $\beta = 450$, and $\alpha = 180$. The nonlinear cost function used is $C(x) = c_1x - c_2x^2$, where $c_1 = 170$ and $c_2 = 50$. The assumed group size in *B–D* is $N = 30$.

Discussion

Our results underline several important caveats for experiments on microbes. In pairwise invasibility experiments, it is important to realize that because a producer may be unable to invade cheats when at low frequencies, it does not necessarily mean it cannot be stably maintained

at higher frequencies. So even if cheats invade cooperators but not the reverse, coexistence at intermediate levels may still be possible. After the demonstration that a producer is exploited by a cheat, one should also test how producers fare at different starting frequencies. It is possible that

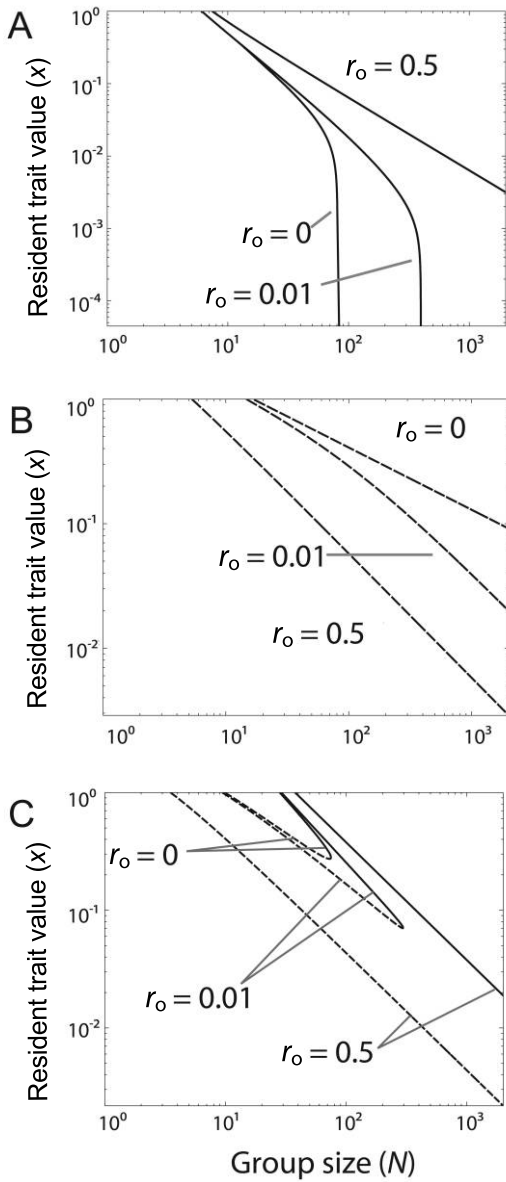


Figure 4: Effect of assortment (positive relatedness) on the evolutionary dynamics of decelerating, accelerating, and sigmoidal benefit functions. Curves correspond to different degrees of others-only relatedness (equal to the values of $\langle \rho \rangle$): $r_o = 0$, 0.01, and 0.5. Solid lines indicate attracting (convergence-stable) singular strategies, while dashed lines indicate singular strategies that are repelling (not convergence stable). The benefit and cost functions here are the same as in figure 2.

above some starting frequencies, producers will achieve intermediate abundances but below this threshold will be lost.

Our analysis also points to the potential necessary considerations of varying density in microbe experiments. If laboratory results of experiments using well-mixed cul-

tures do not accord with natural settings, differences in density in addition to relatedness should be explored. For instance, figures 3 and 4 show that density in addition to relatedness can be crucial to the qualitative evolutionary dynamics. In figure 3, in the simplest case with no assortment, group size and initial conditions determine whether all nonproducers, all cheats, or some combination will be the evolutionary endpoint; as equation (3) shows, group size can also determine whether a uniform level of intermediate investment is the evolutionary endpoint or whether the population splits into a dimorphism. Microbe experiments are characterized by bacterial populations at high densities in nutrient-rich and enemy-free environments, which can be vastly different from many natural environments, and these differences must be carefully considered.

In this article we focused primarily on the shapes of benefit functions in social evolution. A significant part of our consideration was the evolutionary repeller that can

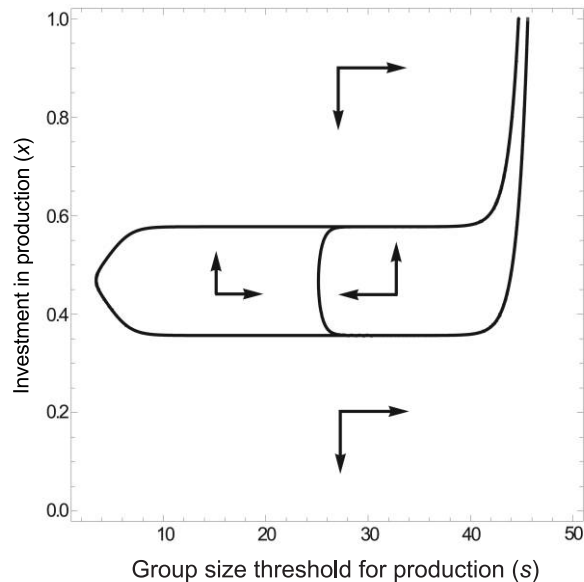


Figure 5: Joint evolution of the public-goods production trait x and the group-size threshold s above which the production is expressed. Benefits and costs are as in figure 2C. At each generation, a group is formed of size 5 or size 45 with equal probability. For very low quorum-sensing thresholds, public-goods production is not affected by changes in group size and evolution inevitably brings the production down to zero. However, for an intermediate range of the quorum-sensing threshold s , public-goods production can be stably maintained. This is true even if the threshold and production are allowed to coevolve, provided that the initial state involves a population with an intermediate quorum-sensing threshold and a sufficiently high expression of production. For other initial conditions, coevolution will bring production down to zero and increase the threshold to arbitrarily large values.

arise in sigmoidal functions. When we considered nonlinear cost functions, it was only to understand their effect on evolutionary branching. However, diminishing cost functions in addition to synergy can possibly lead to the existence of evolutionary repellers (see appendix). Additionally, evolutionary repellers exist in other, noncooperative contexts as well. For instance, dynamics qualitatively similar to those in the sigmoidal case have been demonstrated in epidemiological contexts involving trade-offs in parasite transmission strategies (Ferdynand and Godelle 2005).

Whenever synergy exists, the benefit curve will most likely be sigmoidal because benefits cannot accelerate indefinitely. The most significant qualitative difference between the diminishing functions that are typically assumed and sigmoidal functions is that the latter often permits the creation of an evolutionary repeller, making nonproduction locally stable (fig. 2C). Once the resident trait value has surpassed this repeller, public-goods production is stable, and we have shown that gradual evolution in addition to loss of function mutations can lead to a coexistence between producer and nonproducer. But how does the population cross the repeller from below, allowing cooperation to be established? Kin selection theory offers one explanation. As figure 4 shows, as assortment and group size increase, the repeller becomes arbitrarily close to zero, lowering the hurdle to production. With sufficiently high relatedness, cells in high densities do not need to share the spoils among many genotypes, and thus cooperation can be maintained at arbitrarily high densities. This assortment, along with a jump in phenotype space through mutation or by acquisition of mobile genetic elements, may allow the repeller to be overcome (Smith 2001; Nogueira et al. 2009; Rankin et al. 2011). Even after this crossover, the evolutionary trap is always present. If low-density populations exist for enough time, as is common at some point for bacteria that live in diverse environments, the cooperative trait value can drop below the value of repeller and be lost forever.

One mechanism with which to avoid the evolutionary trap created by synergy is density-dependent gene regulation or quorum sensing. The evolutionary advantage of quorum sensing is usually phrased as limiting the expression of particular genes to certain cellular densities. Cells that express genes only at high densities, when they receive a benefit, gain a fitness advantage over a nonregulated counterpart. We have shown that quorum sensing can prevent nonproducers from invading when density is too low, limiting the cheat load that could otherwise sink cooperation. The density-dependent increase in fitness may be nonsynergistic (Brookfield 1998; Brown and Johnstone 2001; Czárán and Hoekstra 2009) due to, for instance, reduced loss of secretions into the environment at high cellular densities. In contrast, figure 5 shows that when

the regulated trait is synergistic, this effect can prevent the trait from falling below the evolutionary repeller. In either scenario, quorum sensing may help to maintain public-goods production in the population by upregulating it only when it is favored by natural selection. However in the synergistic case, quorum sensing also helps to prevent the collapse of cooperation when cells are growing in low-density environments (fig. 5).

Many bacteria spend much of their time at low densities until their environment changes, for example, upon entering a host. By restricting expression to periods of high density, cooperation is not diminished when density is low for relatively long periods of time. Although low density can cause declines in production regardless of the cause of group augmentation, in the synergistic cases (fig. 2B, 2C), the situation is especially dire. Restricting expression to when population sizes are high effectively bypasses the synergistic portion of the benefit function and, consequently, the cooperative trap (below which cooperation may not recover) is avoided (fig. 5). There are several adaptive explanations for autoinduction sensing, including to sense density, to sense diffusion properties, and even to sense the presence of other strains of bacteria (Brookfield 1998; Redfield 2002). Whatever the mix of adaptive functions selecting for quorum sensing, the prevention of widespread cooperative collapse may be an important consequence. However, we also see in figure 5 that quorum sensing does little to help cooperation initially overcome the repeller. In fact, at very low levels of production (under the evolutionary repeller), the quorum-sensing threshold may actually increase and make stable cooperation even more difficult to achieve. Thus, quorum sensing is initially unhelpful in crossing the repeller, but it may be crucial for the maintenance of cooperation by preventing a population from slipping into the trap after cooperation has already been established.

It would be interesting to integrate our work into a more demographically sophisticated model. Because groups were reformed at each generation in our model, successful individuals replaced other individuals drawn from the entire population. This is different from more realistic models that feature limited dispersal in viscous populations (Wright 1949; Hamilton and May 1977; Rousset 2004). Limited dispersal causes competition among patchmates, and this can have major effects on evolution (Hamilton and May 1977; Taylor 1992). Equation (6) indicates that higher variance in the degree of assortment among groups in a population can make the conditions for evolutionary branching more narrow; this would be interesting to study further in the context of its effects on evolutionary branching in various dispersal regimes (Ajar 2003). Another possible consideration for future work is that in our model, the individual traits did not affect re-

latedness. However, feedback between the trait and relatedness is possible, as in the classic example of the evolution of dispersal rates (Hamilton and May 1977; Rousset and Ronce 2004) where dispersal rate affects relatedness and vice versa (Lion and van Baalen 2008; Lehmann and Rousset 2010). This could also occur when the patch size increases with public-goods production: if a patch has a higher carrying capacity, one that is dependent on cooperation within the patch, synergistic functions may lead to interesting dynamics.

On a more biological note, we reiterate that a particular microbe may best be represented by different functional forms from those described here. For instance, we did not consider the possibility that in high densities, fewer molecules are lost into the environment, which would also mean that group benefits increase at higher densities. In addition, some microbes have preferential access to their own public-goods products, even when in a well-mixed environment (Gore et al. 2009). Future work could, for example, allow producers to get a benefit that is greater than the equal share of production they received in this model. However, even in microbes that have properties different from those specified here, synergy will likely play a prominent role. Finally, the interplay between production and regulation in our model made simplified assumptions about the evolution of autoinduction thresholds; in reality, a particular quorum-sensing network can regulate many traits rather than only one. Among many traits, the quorum-sensing trait will be likely be more stable. The evolvability of various features of quorum sensing (in this case, the group size at which autoinduction occurs) has not received significant attention in the literature; however, there is evidence that various aspects of quorum sensing are evolvable (Ansaldi and Dubnau 2004; Ichihara et al. 2006; Sandoz et al. 2007). Future work integrating molecular details into the evolution of these thresholds could be very interesting.

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