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Report

Cooperation Peaks at Intermediate Disturbance

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Summary

Explaining cooperation is a challenge for evolutionary biology [1, 2]. Surprisingly, the role of extrinsic ecological parameters remains largely unconsidered. Disturbances [3, 4] are widespread in nature and have evolutionary consequences [5]. We develop a mathematical model predicting that cooperative traits most readily evolve at intermediate disturbance. Under infrequent disturbance, cooperation breaks down through the accumulation of evolved cheats. Higher rates of disturbance prevent this because the resulting bottlenecks increase genetic structuring (relatedness [6–8]) promoting kin selection for cooperation. However, cooperation cannot be sustained under very frequent disturbance if population density remains below the level required for successful cooperation. We tested these predictions by using cooperative biofilm formation by the bacterium *Pseudomonas fluorescens* [9, 10]. The proportion of biofilm-forming bacteria peaked at intermediate disturbance, in a manner consistent with model predictions. Under infrequent and intermediate disturbance, most bacteria occupied the biofilm, but the proportion of cheats was higher under less frequent disturbance. Under frequent disturbance, many bacteria did not occupy the biofilm, suggesting that biofilm dwelling was not as beneficial under frequent versus intermediate disturbance. Given the ubiquity of disturbances in nature, these results suggest that they may play a major role in the evolution of social traits in microbes.

Results and Discussion

We developed a simple mathematical model describing the evolution of bacterial cooperation in an environment periodically disturbed by mass-mortality events. Groups of bacteria are founded by one bacterium, or a small number of clonal cells, and these cells undergo exponential growth. Two social strategies are genetically encoded by alternative alleles, so that bacteria can either be cooperators (which contribute a public good to their group) or cheats (which make no such contribution but free-ride on the public goods of others). We consider that cooperation carries a direct growth cost (c) for the cooperative lineage, and if the number of cooperators is above a threshold (τ), it also provides a growth benefit (b) for all members of the group. We also assume loss-of-function mutations arise (at rate μ) and transform cooperators into cheats. After a period of growth (duration T), disturbance destroys all groups and new groups are founded by singleton bacteria (or small numbers of clonal cells), picked at random from the population at the time of disturbance. A full model description and mathematical analysis are presented in the Supplemental Data available online. More realistically, resource competition will lead to deviation from exponential growth, and the group benefits of cooperation will usually be a continuous, rather than step, function of cooperator density [11]. The model considered here is more tractable because of its simplicity, but it is intended only for qualitative illustration.

We found that at high disturbance (low T), cheating predominates in bacterial populations (Figure 1). This arises because the threshold cell density above which cooperation provides a group benefit is never reached by the groups of bacteria, their growth continually being interrupted by mass-mortality events. We also found that cheating predominates at low disturbance (large T; Figure 1). Here, groups grow beyond the threshold size required for cooperation to be helpful but are eventually overrun from within by cheating cells that arise through loss-of-function mutation and that enjoy a within-group selective advantage. Only with intermediate levels of disturbance (medium T) can cooperation predominate (Figure 1). Here, disturbance is sufficiently infrequent for allowing groups to reach the threshold size beyond which cooperative collaboration yields a benefit, and yet disturbance is sufficiently frequent for maintaining genetic structuring (relatedness [6–8]), which gives a kin-selected benefit for cooperation.

Next, we investigated the impact of disturbance frequency on the evolution of cheats experimentally by using the cooperative trait of biofilm formation in *Pseudomonas fluorescens* [10, 12]. When propagated in spatially heterogeneous environments (a static glass microcosm containing nutrient-rich medium [12]), populations

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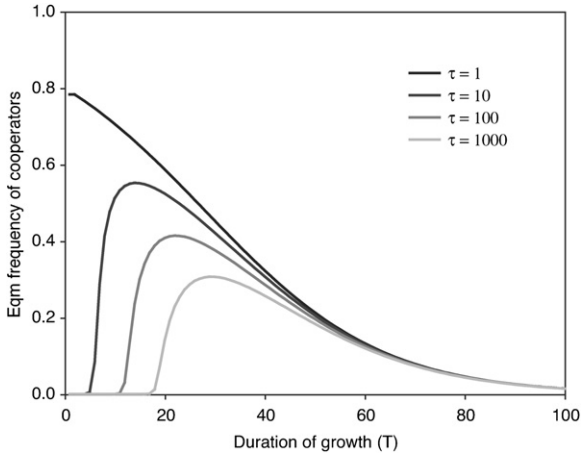

Figure 1. Theoretical Predictions for the Equilibrium Frequency of Cooperation over a Range of Disturbance Rates

We predict that cooperation will not evolve when disturbance rates are high (short-growth duration; low *T*) and nor will it evolve when disturbance rates are low (long-growth duration; large *T*). Cooperation is therefore restricted to environments with intermediate rates of disturbance (medium-growth duration; medium *T*). Illustrative numerical solutions are given for a range of density thresholds (τ), the relative size of the group required for cooperation to provide a benefit. Other parameters are cooperative growth benefit b = 0.1, growth cost c = 0.05, and mutation rate μ = 0.01.

of the ancestral smooth (SM) *P. fluorescens* genotype rapidly diversify and thus generate by mutation a range of niche-specialist genotypes that are maintained by negative-frequency-dependent selection [12]. The wrinkly-spreader (WS) morph is ecologically dominant [13, 14], forming a biofilm at the air-broth interface through constitutive overproduction of cellulosic polymer [15]. Although overexpression of cellulosic polymer is individually costly (as demonstrated by the reduced exponential growth rate of WS relative to SM [10, 16]), its production provides a group benefit to WS because colonization of the air-broth-interface niche allows improved access to oxygen, a limiting resource [10]. Clonal WS biofilms have been found to be susceptible to rapid invasion by SM genotypes that arise by mutation from WS over the course of several days [9, 10]. In this context SM are cheats, gaining the benefit of inhabiting the air-broth interface but making no contribution to the integrity of the biofilm, which is significantly weaker in the presence of cheating SM genotypes [10]. Note that SM can also inhabit the less productive liquid phase of the microcosm and therefore can in principle coexist with WS with or without cheating to inhabit biofilm.

Four independent WS genotypes were isolated from separate adaptive radiations. Initially, isogenic populations of each were then propagated under five disturbance regimes. Disturbances were nonspecific mass-mortality events: After thorough homogenization, 99.9% of the population was discarded and the remaining 0.1% ($\sim 10^6$ cells) was transferred to a fresh microcosm. Experiments were performed in static microcosms over a 16 day period during which populations were disturbed daily, every second day, fourth day, eighth day, or not at all. After 16 days, populations were homogenized and plated onto agar, and the frequency of SM and WS colonies determined. The proportion of WS cooperators within populations displayed a unimodal relationship with disturbance frequency, peaking at intermediate rates (Figure 2; cooperator proportion highest under 4 day disturbance; founding genotype, $F_{1,16} = 3.01$, p = 0.1; linear term, $F_{1,16} = 1.9$, p = 0.19; negative quadratic term, $F_{1,16} = 54.6$, p < 0.0001). Thus, intermediate frequency disturbance can forestall the breakdown of cooperation.

Increases in SM density could have arisen through two mechanisms: First, SM could have inhabited the biofilm as nonproducing cheats; second, SM could have left the biofilm entirely and invaded the broth phase of the microcosms. To address this, we sampled and plated the broth phase of each population prior to homogenization. We then subtracted this value from the total number of SM in the population to calculate the density of the biofilm-inhabiting portion of the population. Only under the highest frequency of disturbance did the majority of SM inhabit the broth phase; under all other disturbance regimes, the majority of SM inhabited the biofilm as nonproducing cheats (Figure 3; linear regression of the proportion of total SM inhabiting the biofilm as cheats against disturbance regime, $F_{1,18} = 8.59$, *Rsq* = 0.3, p = 0.008). Therefore, in agreement with our model predictions, there was strong selection for the evolution of cheats within the biofilm under infrequent disturbance. By contrast, cooperative biofilm formation per se was less favored under frequent disturbance; here, WS cellulose production, although costly, is likely to have conferred little benefit. Thus, SM prospered because of their inherent growth-rate advantage [10, 16].

To assess whether the apparent reduction in benefit of biofilm formation under frequent disturbance was the result of lower densities, we measured total population densities on day 16 of the experiment. Population density displayed a unimodal relationship with disturbance frequency (Figure 4; founding genotype, $F_{1,16} = 0.24$, p = 0.63; linear term, $F_{1,16} = 41.1$, p < 0.0001; negative quadratic term, $F_{1,16} = 89.2$, p < 0.0001), and in line with our model predictions, density was significantly lower in populations disturbed daily compared to every

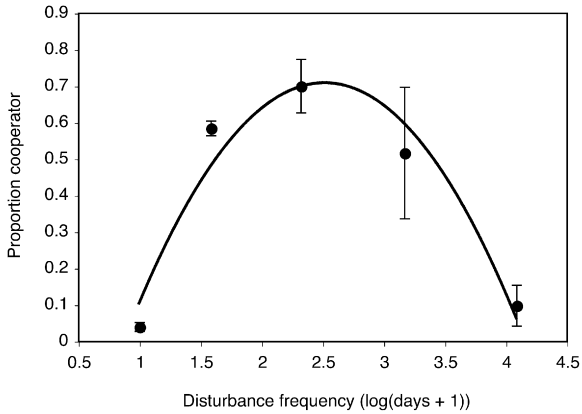

Figure 2. The Effect of Disturbance Frequency on the Proportion of Cooperators

Dots represent mean proportion ± SEM of the population with WS colony morphology on day 16 of the experiment.

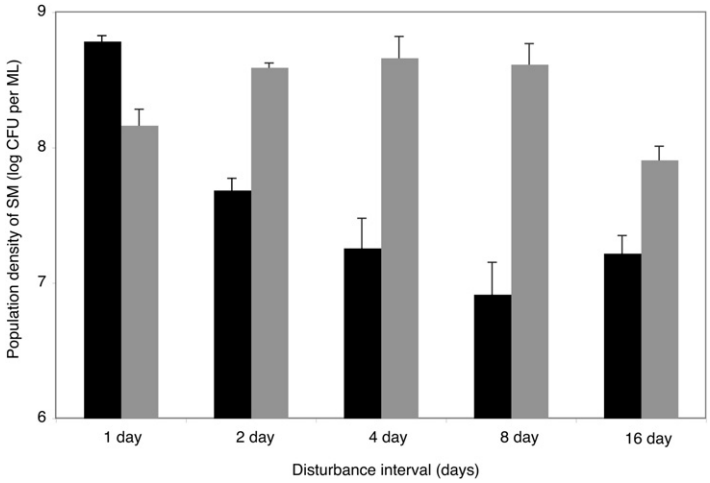

Figure 3. The Densities of SM Inhabiting the Broth and Biofilm Phases

Bars represent mean density + SEM of SM population inhabiting the broth phase (black) or biofilm phase (gray) of microcosms on day 16 of the experiment.

fourth day (t test comparing log population density under 1 day and 4 day disturbance; $t = 56.3$, df = 3, $p < 0.0001$). Thus, under frequent disturbance it is likely that the population did not have time to grow to capacity before each subsequent disturbance, whereas under infrequent disturbance, the population suffered from starvation and cells began to die.

Despite the simplicity of our model, it successfully predicts de novo evolution of social traits in a bacterial system. Moreover, we believe these results are likely to have broad relevance to understanding microbial cooperation. Our model predictions rest on three biological assumptions. First, that cooperation is less useful at low density; without this, cooperation could be selectively favored at high disturbance frequencies. The threshold assumption of our model is not crucial to the observed effect; rather, this effect relies on density dependence whether in the form of a continuous or step function (data not shown). Such positive density-dependent regulation of growth rate, termed the "Allee Effect" [17], has been observed in a wide range of natural systems [18], including those where social traits are important for survival [19]; indeed, fruiting body formation by the social bacterium *Myxococcus xanthus* has been shown to display positive density dependence [20]. It is inevitably true that the benefit of biofilm formation will have density dependence, simply because many of biofilm dwelling's benefits, such as resistance to environmental stresses and communication, rely on high-density conglomerations of cells [21, 22]. In our experimental system, a critical number of cells are required to attach to the glass at the air-broth interface to anchor the biofilm in place. It also seems likely that the group benefit of public-good cooperation in general will be density dependent [23]. For example, the encounter rate between the producers of extracellular-scavenging molecules and the molecules themselves will increase with cell density, resulting in less molecules being wasted. Similarly, a large number of individuals may be required to produce sufficient toxins or antibiotics to kill prey or competitors or to successfully establish an infection [24]. In further support of this, the production of many such public-good-type products is regulated in a density-threshold-dependent manner by quorum sensing [25, 26].

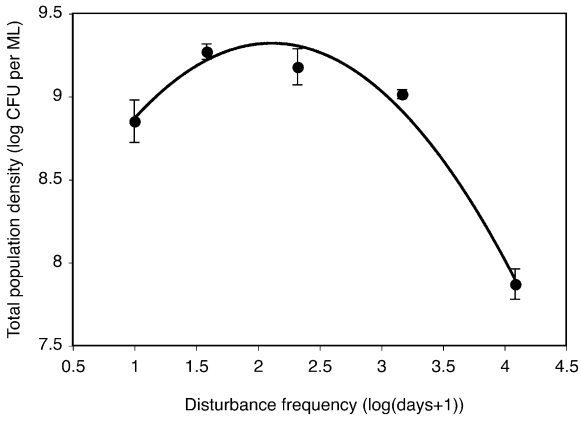

Figure 4. The Effect of Disturbance Frequency on Population Density

Dots represent mean ± SEM population density on day 16 of the experiment.

The second assumption is that disturbances cause population bottlenecks, which result in increased genetic structuring (relatedness [6–8]) such that cooperators are less likely to be associated with cheats after a disturbance. This allows for kin selection to favor cooperation and will of course depend on the disturbance's magnitude, which is likely to be impossible to quantify in natural populations of bacteria. However, the highly clonal population structure of bacteria would suggest that most groups are founded by a single or very few individuals [27].

The third assumption is that mutations from cooperation to cheat occur at a higher frequency than vice versa. This is indeed likely to be the case in microbes where many characterized cheating strategies involve loss-of-function mutations [28–30], which are more likely to occur than subsequent gain-of-function mutations, although such mutations are possible [28].

There are, however, details of our experimental system that are not explicit in our model, as is inevitable when attempting to describe a general effect. First, disturbances in our experiment are linked with replenishment of resources; this is the case with disturbances

in general [4]. This may have led to an additional benefit to SM through its growth-rate advantage over WS when resources are abundant [10]. This would have acted in concert with the model prediction that cooperative biofilm formation per se is likely to be disfavored under frequent disturbance because of low population density; here, cooperation is individually costly but confers reduced group benefit. Second, we observed cell death as a result of starvation in the highly undisturbed microcosms; thus, the domination of 16 day cultures by SM could have resulted from the lower death rate of SM compared to WS. An elevated WS death rate may represent a further direct pleiotropic cost of this cooperative phenotype [16], or a further deleterious consequence of social cheating by SM [10]; however, the action of an unrelated parameter cannot be ruled out. Unfortunately, there is no clear experimental test to tease apart these possibilities in this experimental system. It is likely that these specifics of the experimental system work together with the general mechanisms predicted by our model to explain our experimental results; future work should therefore investigate the effect of disturbance on other microbial social traits.

Intermediate disturbance has been implicated in the maintenance of ecological diversity by its prevention of the dominance of ecosystems by the most competitive species (the "intermediate-disturbance hypothesis" [31]) and mediation of the relative productivity of ecological niches [3]. This study demonstrates a further important role for intermediate disturbance. We show that intermediate frequencies of disturbance can create conditions that retard the breakdown of cooperation within a microbial social group in the absence of other, more complex, supporting mechanisms (such as policing [32, 33], punishment [34, 35], reciprocity [36], or character displacement [9]). Thus, kin-selected cooperation within microbial populations may be more robust than previously thought under intermediate disturbance, extending the conditions under which cooperation can be maintained. These results highlight the importance of integrating ecological and evolutionary perspectives in order to understand the maintenance of social traits [37–40].

Experimental Procedures

Isolating WS Genotypes

Four replicate microcosms (30 mL glass universal containing 6 mL of King's B nutrient media) were inoculated with *Pseudomonas fluorescens* SBW25 to a total of approximately 10^7 cells. These were statically incubated for 6 days at 28°C, after which time all populations were vortexed and an aliquot was diluted and plated onto KB agar. A single wrinkly-spreader colony was then isolated from each population for further study and stored at −80°C in 20% glycerol.

Disturbance-Selection Experiment

Populations were initiated with 10^7 cells of one of the isolated WS genotypes grown for 18 hr under shaken conditions. These were then propagated under one of the following disturbance regimes: 6 μL of culture was transferred to a fresh microcosm every 1 day, 2 days, 4 days, 8 days, or not at all during a 16 day period. After 16 days, the broth phase of each population was sampled, and then populations were homogenized and sampled. Samples were then plated onto agar and the frequencies of WS and SM colonies were counted.

Supplemental Data

Supplemental Data include additional Discussion and are available with this article online at http://www.current-biology.com/cgi/content/full/17/9/761/DC1/.

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Supplemental Data
Cooperation Peaks at Intermediate Disturbance

Michael A. Brockhurst, Angus Buckling, and Andy Gardner

Supplemental Discussion

We consider a simple model in which groups of bacteria are founded either by a single cell or a small number of clonal cells. The growth of the cell lineage is exponential, with the baseline rate set to unity for convenience. This is made possible by the choice of an appropriate unit of time, which is arbitrary, and it is helpful that durations of time can now be expressed in terms of pure (dimensionless, or unitless) numbers. Cooperation provides a benefit at the group level when the number of cooperators (expressed as a multiple of the initial group size) exceeds τ, a dimensionless parameter. Above this threshold, cooperators pay an individual growth-rate cost c, and all members of the group receive a growth-rate benefit b. Below the threshold, cooperators pay the cost c, but there is no group benefit. Additionally, with rate μ, individual cooperators are transformed into cheats through loss-of-function mutation. Finally, groups are destroyed by disturbance after a growth interval of duration T. During this interval, at any time t the change in the number of cooperators (x) and the number of cheats (y)—expressed as multiples of the initial group size—is described by the following dynamical equations:

$$\frac{dx}{dt} = (1 - c - \mu)x_t \tag{1A}$$

$$\frac{dy}{dt} = y_t + \mu x_t \tag{1B}$$

when $x_t < \tau$, and:

$$\frac{dx}{dt} = (1 + b - c - \mu)x_t \tag{2A}$$

$$\frac{dy}{dt} = (1 + b)y_t + \mu x_t \tag{2B}$$

when $x_t \geq \tau$.

Because groups are formed by a single bacterium or a small number of clonal cells, we can discriminate "cooperative" groups and "cheating" groups according to the initial social behavior exhibited by the group's members. If the global frequency of cooperators at the moment of disturbance at the end of the growth phase is p, then the proportion of cooperative groups in the next growth phase will be p and the proportion of cheating groups will be $1 - p$. Thus, disturbance impacts on the population density and degree of genetic structure but does not affect the population frequency of cooperators. Note that if cooperative groups cannot reach the threshold number of cooperators (τ) within the duration of the growth interval (T), then cooperation is disfavored (because there is no benefit of cooperation), and over repeated rounds of growth and disturbance it will decline in frequency and ultimately be lost from the population. This happens when the number of cooperators at time T is less than τ, i.e., from solving Equation 1A:

$$T < \frac{\ln(\tau)}{1 - c - \mu}. \tag{3}$$

Thus, when inequality (3) is satisfied (low T, high disturbance), cooperation is never favored, and the equilibrium frequency of cooperation is $\hat{p} = 0$.

Assuming that inequality (3) is not satisfied, then there is a possibility that cooperation can evolve. To see when this will occur, we need to follow the growth dynamics of the two group types separately. Cheating groups show simple growth because they only ever contain cheats (there is no possibility of cooperation arising by mutation), and hence they never cross the threshold number of cooperators. Thus, solving Equations 1A and 1B, we find that the number of cheats in such a group at time T is simply:

$$y_T = e^T. \tag{4}$$

Cooperative groups are more complicated. At the time that the threshold number of cooperators is reached, there are τ cooperators in such groups. This occurs when t is equal to the RHS of inequality (3), which we shall denote T^*, and hence solving Equation 1B we have:

$$y_{T^*} = \frac{\mu}{c + \mu}\tau\left(\tau^{\frac{c+\mu}{1-c-\mu}} - 1\right). \tag{5}$$

Solving Equations 2A and 2B, the number of cheats and cooperators at time T in each group founded by cooperators is:

$$x_T = e^{(1+b-c-\mu)T}\tau^{-\frac{b}{1-c-\mu}} \tag{6}$$

$$y_T = \frac{\mu}{c + \mu}e^{(1+b-c-\mu)T}\left(e^{(c+\mu)T} - 1\right)\tau^{-\frac{b}{1-c-\mu}}. \tag{7}$$

Thus, the population frequency of cooperators at time T is given by the total number in the population divided by the total number of individuals in the population at time T:

$$p' = \frac{pe^{(1+b-c-\mu)T}\tau^{-\frac{b}{1-c-\mu}}}{p\left(e^{(1+b-c-\mu)T}\tau^{-\frac{b}{1-c-\mu}} + \frac{\mu}{c+\mu}e^{(1+b-c-\mu)T}\left(e^{(c+\mu)T} - 1\right)\tau^{-\frac{b}{1-c-\mu}}\right) + (1-p)e^T}. \tag{8}$$

Note that $p' \to 0$ as $T \to \infty$, and hence in the limit of large T (low disturbance) the equilibrium frequency of

cooperation is zero ($\hat{p} \to 0$ as $T \to \infty$). The combination of this with our earlier result that low T (high disturbance) disfavors cooperation shows that if cooperation is ever to be favored, it must be at intermediate levels of disturbance.

Equation 8 provides a recursion describing how the frequency of cooperators across the population changes from one round of growth and disturbance to the next. Expressions describing the dynamics and equilibria implicitly can be derived analytically, but for explicit results, we require numerical methods. Some numerical results, showing how the equilibrium level of cooperation varies as a function of the rate of disturbance, are given in Figure 1.

It should be noted that our simple model assumes exponential growth, so that there is no competition between individuals during growth, and all competition occurs at the population level during disturbance when only a few individuals survive the catastrophe. More generally, limiting resources could generate local competition within groups during the growth period, and this may reduce the productivity of cooperative groups and thus inhibit the evolution of cooperation. Resource limitation is most likely to be experienced under infrequent disturbance, where resource competition will be strongest. Disturbances can reduce resource competition in our experimental system as well as most natural systems by reducing population density and providing an input of nutrients and as such could promote cooperation. This is analogous to the competition-easing effect of dispersal, which has been much studied [S1–S3]. However, too high a frequency of disturbance, leading to a surfeit of resources, could disfavor cooperative public-good contributions that work to increase resource availability for the group; i.e., cooperation is simply not needed. Hence, competition effects might also lead to cooperation being favored only at intermediate-disturbance rates. Thus, there are interesting possibilities for resource competition in this system, which might profitably be considered in the future, although we emphasize that the basic qualitative prediction of a unimodal relation between disturbance frequency and the level of cooperation holds even in the absence of resource competition.

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