

LIMITED DISPERSAL, BUDDING DISPERSAL, AND COOPERATION: AN EXPERIMENTAL STUDY

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Numerous theoretical studies have investigated how limited dispersal may provide an explanation for the evolution of cooperation, by leading to interactions between relatives. However, despite considerable theoretical attention, there has been a lack of empirical tests. In this article, we test how patterns of dispersal influence the evolution of cooperation, using iron-scavenging in the bacterium *Pseudomonas aeruginosa* as our cooperative trait. We found that relatively limited dispersal does not favor cooperation. The reason for this is that although limited dispersal increases the relatedness between interacting individuals, it also leads to increased local competition for resources between relatives. This result supports Taylor's prediction that in the simplest possible scenario, the effects of increased relatedness and local competition exactly cancel out. In contrast, we show that one way for cooperation to be favored is if individuals disperse in groups (budding dispersal), because this maintains high relatedness while reducing local competition between relatives (relatively global competition).

KEY WORDS: Experimental evolution, kin selection, local competition, microorganisms, relatedness, social evolution.

Explaining cooperation is fundamental for understanding the evolutionary transitions from associations of replicator molecules to single-celled organisms to complex animal societies (Maynard Smith and Szathmari 1995; Hamilton 1996; Frank 2003; West et al. 2007a). Why should an individual carry out a costly cooperative behavior that benefits other individuals? Hamilton's (1963, 1964) theory of kin selection provides an explanation for how cooperation can be favored if interacting individuals are likely to share the gene for the cooperative behavior, for example, if they are relatives. By helping a close relative reproduce, an individual passes its genes to the next generation, although indirectly. This theory is encapsulated in a pleasingly simple form by Hamilton's (1963, 1964) rule, which states that altruism is favored when $rb - c > 0$; where c is the fitness cost to the altruist, b is the fitness benefit to the beneficiary and r is their genetic relatedness. Hamilton (1964, 1972) originally suggested that a high relatedness could arise in two ways: (1) direct kin recognition between individuals, or (2) limited dispersal (population viscosity) can keep relatives together, and so could favor indiscriminate altruism.

The possible role of limited dispersal in favoring cooperation has attracted much theoretical attention (Hamilton 1964, 1971, 1972, 1975; Pollock 1983; Grafen 1984; Murray and Gerard 1984; Goodnight 1992; Kelly 1992; Nowak and May 1992; Queller 1992; Taylor 1992a,b; Wilson et al. 1992; Kelly 1994; Nowak et al. 1994; Queller 1994; Frank 1998; van Baalen and Rand 1998; Mitteldorf and Wilson 2000; Griffin and West 2002; West et al. 2002b; Hauert and Doebeli 2004; Rousset 2004; Gardner and West 2006; Killingback et al. 2006; Lehmann and Keller 2006; Ohtsuki et al. 2006; Traulsen and Nowak 2006; Grafen 2007a,b; Lehmann et al. 2006, 2007a,b; Taylor et al. 2007; Alizon and Taylor 2008; Grafen and Archetti 2008; Johnstone and Cant 2008; Lion and van Baalen 2008; Shaw and Gardner 2008). Limited dispersal could potentially be a very important mechanism because it does not require any mechanism for kin discrimination (Hamilton 1964), and so could occur even in the simplest of organisms. This has led to limited dispersal being suggested as an important driving force in numerous specific cases, including the evolution of ATP pathways (Pfeiffer et al. 2001), the

transition from single cells to multicellular organisms (Michod 1997; Michod and Roze 2001; Pfeiffer and Bonhoeffer 2003), cooperative behaviors in bacteria and other micro-organisms (Crespi 2001; West et al. 2006), the evolution of eusociality in social insects (Bourke and Franks 1995), cooperative breeding in vertebrates (Griffin and West 2002), lower levels of aggression between competitors (Murray and Gerrard 1984), and even mutualistic cooperation between species (Doebeli and Knowlton 1998; Bever and Simms 2000; West et al. 2002a).

In contrast, other theoretical models have suggested that limited dispersal will not necessarily favor the evolution of cooperation (Kelly 1992; Queller 1992a; Taylor 1992a,b; Wilson et al. 1992). The reason for this is that limited dispersal can also lead to local competition between kin, which selects against cooperation (West et al. 2002b). In two pivotal papers, Taylor (1992a,b) built upon previous simulation work (Wilson et al. 1992) to show that, in the simplest possible scenario, the effects of dispersal on relatedness and competition exactly cancel, and so the dispersal rate has no influence on selection for cooperation. The reason for that is although low rates of dispersal result in genealogical closeness (and hence genetic relatedness) between social partners, the extra progeny created by cooperation will compete locally for resources, and so cooperative groups will be characterized by more intense competition. Put simply, there is no point helping a sibling if its increased reproductive success comes at the cost of another sibling. Since then, a large body of theory has been developed, showing that the relative importance of the relatedness and competition effects, and hence whether limited dispersal favors cooperation, will depend upon biological details (Goodnight 1992; Kelly 1992; Kelly 1994; Queller 1994; Frank 1998; van Baalen and Rand 1998; Mitteldorf and Wilson 2000; Taylor and Irwin 2000; West et al. 2002b; Le Galliard et al. 2003; Gardner and West 2006; Lehmann et al. 2006; Grafen 2007a,b; Lehmann 2007a,b; Johnstone and Cant 2008; Shaw and Gardner 2008).

Despite considerable theoretical attention, there has been a lack of empirical work testing the predictions for how population structure should influence the evolution of cooperation. Indeed, Hamilton (1996) left it as a major unsolved problem. An exception is a previous experimental study of Griffin et al. (2004), which showed that higher relatedness favored cooperation, and that increased local competition between relatives selected against cooperation. However, although this study showed that relatedness and competition can both matter, these two factors were manipulated independently and it did not test how their relative importance varies with the dispersal rate, which was equally high in all treatments. In natural populations, relatedness and the scale of competition emerge as a result of population demography, and will not usually be independent (Taylor 1992a,b).

Our aim here is to provide the first experimental tests of: (1) how the individual dispersal rate influences selection for cooper-

ation, and (2) whether selection for cooperation can be increased if individuals disperse in groups of relatives, termed budding dispersal (Goodnight 1992; Gardner and West 2006; Lehmann et al. 2006). Budding dispersal can favor cooperation because it combines relatively high relatedness and relatively low competition between social partners (Gardner and West 2006). High relatedness is maintained because individuals disperse in groups of relatives. Kin competition is low because more productive social groups give rise to more dispersing buds, which compete globally (at the scale of the whole population) for resources. Dispersal in small kin groups has been observed in a number of cooperative species, including unicellular microorganisms (Pfeiffer and Bonhoeffer 2003), social insects (Peeters and Ito 2001), cooperative breeding birds (Williams and Rabenold 2005; Sharp et al. 2008), humans (Haldane 1932), nonhuman primates (Bradley et al. 2007), bats (Metheny et al. 2008), and other mammals (Clutton-Brock 2002).

We test these predictions using an experimental evolution approach with *Pseudomonas aeruginosa* bacteria. The social trait that we examine is the production of iron-scavenging molecules termed siderophores (Budzikiewicz 2001; Cornelis and Matthijs 2002; Griffin et al. 2004; Visca et al. 2007). Iron is a major limiting factor for bacterial growth, because most iron in the environment is in the insoluble Fe(III) form and, in the context of bacterial parasites, is actively withheld by hosts (Guerinot 1994; Ratledge and Dover 2000; Miethke and Marahiel 2007). We have previously shown that the production of the primary siderophore (pyoverdine) is a cooperative trait subject to kin selection. Specifically, pyoverdine production is metabolically costly to the individual cell, but provides a benefit to the local group (Griffin et al. 2004; Harrison et al. 2006; Ross-Gillespie et al. 2007). We construct replicate populations, divided into 12 subpopulations, each of which we initiate with a mix of cooperators, which produce pyoverdine and noncooperating mutants (cheats), which do not produce pyoverdine. We then experimentally manipulate the type of dispersal between populations, to simulate relatively limited dispersal, relatively high dispersal, and budding dispersal (Fig. 1). The consequences of the different dispersal regimes for the relative fitness of cooperators and cheats were then followed. Finally, we built a population genetic model that could be parameterized with our experimental data, to elucidate the underlying selective forces.

Material and Methods

STRAINS

We used *P. aeruginosa* strain ATC 15692 (PAO1) as the pyoverdine (pvd) producer, and strain PA06609 (PAO9) as the pvd-negative cheat. PAO9 is a pvd-mutant derived by UV-mutagenesis from methionine auxotroph PAO6409 (Hohnadel et al. 1986), which in

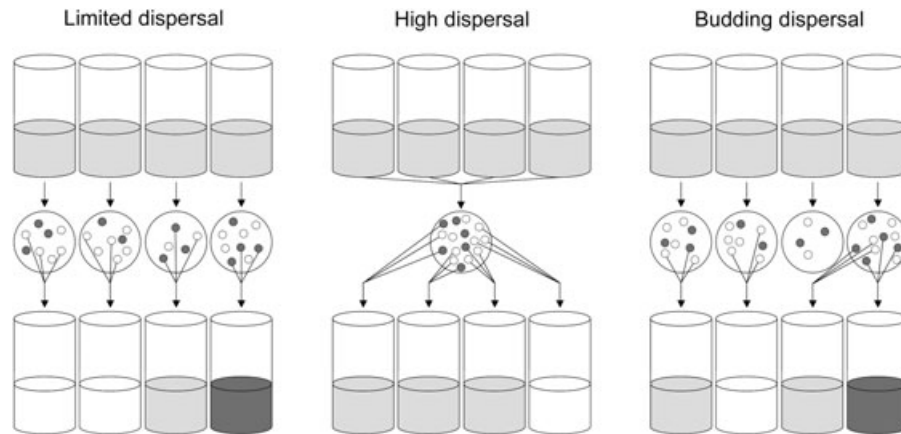


Figure 1. Experimental design manipulating bacteria dispersal under three different regimes (relatively limited, relatively high, and budding dispersal) in populations consisting of 12 subpopulations (for simplicity only four subpopulations per dispersal regime are shown). At the beginning, each subpopulation contained a mix of cooperators and cheats (light gray), whereas subpopulation composition changed over the course of the experiment (dark gray and white symbolize monocultures of cooperators and cheats, respectively). For relatively limited dispersal ($d = 0$), we randomly selected three colonies from each subpopulation that seeded a new subpopulation. For relatively high dispersal ($d = 1$), we randomly chose 36 colonies from a mix of all subpopulations and randomly arranged them in 12 triplets, with each triplet seeding a new subpopulation. For budding dispersal, we randomly chose 12 budding groups among all subpopulations with each budding group consisting of three colonies coming from the same subpopulation and seeding a new subpopulation together. With budding dispersal, individual subpopulations could seed zero, one, or more than one new subpopulation with highly productive subpopulations having a higher probability to seed more than one new subpopulation. We carried out 12 successive dispersal events each after a 24-h competition phase. The whole experiment of 12 dispersal events under three different dispersal regimes was repeated six times.

turn was generated by transposon mutagenesis from PAO1 (Rella et al. 1985). Before experiments started, both strains were grown overnight in 30 mL glass universals containing 6 mL standard King's medium B (KB) in an orbital shaker (200 rpm) at 37°C. Both strains grow to similar cell densities under these conditions (Griffin et al. 2004).

EXPERIMENTAL DESIGN

We experimentally manipulated dispersal behavior by allowing bacteria to either disperse individually within subpopulations (relatively limited dispersal), individually between subpopulations (relatively high dispersal), or as groups between subpopulations (budding dispersal). For each dispersal regime, we initially created a population consisting of 12 subpopulations with half of the subpopulations consisting either of a 2:1 or a 1:2 ratio of cooperators and cheats, resulting in a 1:1 overall population ratio of cooperators and cheats. Each subpopulation (approx. 10^6 cells from overnight KB cultures) was grown in a 30 mL tube containing 6 mL of Casamino acids medium (CAA; 5 g casamino acids, 1.18 g $K_2HPO_4 \cdot 3H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, per litre) supplemented with 20 mM $NaHCO_3$ (sodium bicarbonate) and $100 \mu g mL^{-1}$ human apo-transferrin (Sigma) (Meyer et al. 1996; Griffin et al. 2004). Apo-transferrin, combined with bicarbonate, is a powerful natural iron chelator (Schlabach and Bates 1975). It was used to bind the free Fe(III) in the CAA media, which prevents

nonsiderophore-mediated uptake of iron by bacteria. Subpopulations were then grown for 24 h in a static incubator at 37°C, during which time approximately five to seven generations take place.

After 24 h, subpopulations from the limited and budding dispersal regime were individually plated onto KB agar, whereas equal volumes from each high dispersal subpopulation were mixed before plating onto a single KB plate (Fig. 1). Plates were incubated overnight at 37°C, after which time we quantified the number of PAO1 and PAO9 colony forming units (CFU) on each plate. Colonies of PAO1 and PAO9 differ in their morphology and can also easily be distinguished based on color differences: PAO1 colonies are green due to the presence of fluorescent pyoverdinin molecules, whereas colonies of PAO9 are white due to the absence of pyoverdinin molecules.

We then let bacteria disperse according to the three dispersal regimes (Fig. 1). For relatively limited dispersal, we randomly selected three colonies from each plate that seeded a new subpopulation. For relatively high dispersal, we randomly chose 36 colonies from a mix of all subpopulations and randomly arranged them in 12 triplets, with each triplet seeding a new subpopulation. These two dispersal patterns represent the extremes from Taylor's (1992a,b) dispersal (d) parameter range: $d = 0$ for relatively limited dispersal (no dispersal between subpopulations) and $d = 1$ for relatively high dispersal (complete dispersal between subpopulations). We chose the extremes of the parameter range to maximize

experimental power. For budding dispersal, we randomly chose 12 budding groups each consisting of three colonies among all subpopulations, with the constraint that the three colonies forming a budding group derived from the same subpopulation, and with each budding group seeding a new subpopulation. Importantly, individual subpopulations could contribute zero, one, or more than one budding group to the new generation whereby the likelihood of a subpopulation being chosen was proportional to its CFU. Thus, more productive subpopulations (high CFU) had a higher probability to seed more than one new subpopulation. To be able to compare the effect of dispersal on the evolution of cooperation, we kept the number of founder colonies (three) per subpopulation constant across dispersal regimes. The effects of changing the number of founders are analyzed by Gardner and West (2006), showing that smaller numbers of founders lead to higher within-subpopulation relatedness and to increased probability of cooperation being favored.

For stochastic sampling of subpopulations and type of bacterial strain (cooperator or cheat) we used the random number generator implemented in Excel (Microsoft). To control for initial starting frequencies of cheats and cooperators before the subsequent growth phase in CAA, randomly selected colonies were separately inoculated into 200 μ l KB media on a microtitre plate and grown over night at 200 rpm and 37°C. Cell densities did not differ between strains when grown under these conditions (ANOVA: $F_{1,14} = 0.08$, $P = 0.78$).

Growth in CAA followed by transfer (i.e., dispersal event) was repeated 12 times, allowing between 60 and 84 bacterial generations under experimental conditions. The whole experiment of 12 dispersal events under three different dispersal regimes was repeated six times.

FITNESS OF COOPERATORS

Relative fitness (v) of cooperators in 2:1 and 1:2 ratio subpopulations was calculated as $v = x_2(1 - x_1)/x_1(1 - x_2)$, where x_1 is the initial proportion of cooperators and x_2 is their final proportion. The fitness value of v therefore signifies whether cooperators increase in frequency $v > 1$, decrease in frequency $v < 1$, or remain at the same frequency $v = 1$. As with our previous experiments (Griffin et al. 2004; Ross-Gillespie et al. 2007), data on the relative fitness of PAO1 cooperators in an iron-limited environment confirmed that pyoverdinin production has the characteristics of a cooperative trait that provides a benefit to the local group, but can be exploited by individuals (cheats) that produce less or no pyoverdinin (strain PAO9 in our experiment). The exploitability of pyoverdinin production is best demonstrated in mixed subpopulations in which relative fitness of PAO1 cooperators compared to PAO9 cheats was significantly lower than 1 (for 2:1 ratio of cooperators and cheats, $v = 0.52 \pm 0.02$ (mean \pm SE), one-sample t -tests: $t_{169} = -22.5$, $P < 0.0001$, for 1:2 ratio of cooperators and

cheats, $v = 0.70 \pm 0.02$; $t_{232} = -15.9$, $P < 0.0001$). By contrast, the benefit of pyoverdinin production became obvious when subpopulations consisted only of cooperators or cheats, whereby pure cooperator subpopulations grew to significantly higher densities (CFU = 220.2 ± 6.4) than pure cheat subpopulations (CFU = 160.5 ± 3.2 , two-sample t -test: $t_{590} = 7.6$, $P < 0.0001$). That subpopulations with more cooperators grow to higher densities was also found for mixed cultures in which subpopulations with a 2:1 ratio of cooperators and cheats grew to significantly higher densities (CFU = 226.8 ± 6.3) than subpopulations with a 1:2 ratio of cooperators and cheats (CFU = 174.6 ± 5.7 , two-sample t -test: $t_{401} = 7.1$, $P < 0.0001$). Overall, there was a significant difference in the CFU-counts between subpopulations that consisted of zero, one, two or three cooperators (ANOVA: $F_{3,991} = 43.6$, $P < 0.0001$). Post-hoc pairwise comparisons (P -values adjusted using the false discovery rate control method; Benjamini and Hochberg 1995) revealed that CFU-counts significantly differed between any two subpopulation types ($P < 0.035$), except for subpopulations with two and three cooperators for which there was no significant difference in the CFU-counts ($P = 0.42$).

STATISTICAL ANALYSIS

For each dispersal regime and dispersal event, we determined the proportion of cooperators selected for the next CAA growth phase and used these values as the response variable in our analysis. As each dispersal regime was replicated six times, we obtained 18 independent datapoints that we compared in univariate analyses of variance (ANOVA). Factor levels were reduced from the full model by stepwise deletion (model simplification following Crawley (2002)). Although the distribution of the response variable did not significantly deviate from a normal distribution (Shapiro-Wilk test on logarithmic transformed values: $W = 0.93$, $P = 0.22$), the homogeneity of variances was not satisfied (Bartlett test: $K = 6.44$, $P = 0.040$). Thus, to check the robustness of the results obtained from ANOVA analyses, we also conducted randomization tests. To do so, we first classified the obtained F -value from the ANOVA as the observed F -value (F_{obs}). We then performed 10,000 randomizations of our datapoints and performed an ANOVA and extracted the F -value after each randomization step. This provided us with a distribution of F -values based on our dataset, with the probability of getting F -values greater or equal to the observed F -value ($n_{F \geq F_{\text{obs}}}$) being $P = (1 + n_{F \geq F_{\text{obs}}})/10000$. All statistical computations were conducted using R 2.7.0 (available on <http://www.r-project.org/>).

We further used the number of cooperators and cheats after each dispersal event to calculate the relatedness within subpopulations. Comparing dynamics of relatedness between the different dispersal regimes across dispersal events is important because relatedness is not manipulated directly in our experiment, but rather it is determined by the dispersal regime and, in particular,

is allowed to evolve. A general point here is that the relatedness that matters is that for the cooperative trait being examined, (i.e., whether or not individuals produce pyoverdinin our case) and not relatedness across the whole genome (see Results; Hamilton 1964, Grafen 1985). Relatedness with respect to any trait is formally defined as the ratio of the covariance of genetic breeding value for the trait between social partners and the variance in genetic breeding values, or

$$r = \frac{\text{cov}(x, x')}{\text{cov}(x, x)}, \quad (1)$$

where x is the genetic breeding value of the focal individual, x' is the genetic breeding value of its social partners, and cov is the statistical covariance (i.e., $\text{cov}(x, x') = E(x \times x') - E(x)E(x')$) and $\text{cov}(x, x) = E(x^2) - E(x)^2$, where E denotes an expectation or arithmetic mean; Frank 1998). We may arbitrarily assign cooperators a breeding value of $x = 1$ and cheats $x = 0$; hence, $E(x^2) = E(x) = E(x')$, and $r = (E(x \times x') - E(x)^2)/(E(x) - E(x)^2)$. Noting that subpopulations can exist in four different states according to whether they have been founded by 0, 1, 2, or 3 cooperator colonies, we can reexpress relatedness as

$$r = \frac{\sum_{i=0}^3 \pi_i (i/3)^2 - \left(\sum_{i=0}^3 \pi_i (i/3) \right)^2}{\sum_{i=0}^3 \pi_i (i/3) - \left(\sum_{i=0}^3 \pi_i (i/3) \right)^2}, \quad (2)$$

where π_i is the frequency of subpopulations that are founded by i cooperator colonies. The values of π_i are known for every dispersal event of each replicate of the experiment, allowing us to describe the temporal dynamics of relatedness. Note that, because $r = 0/0$ if $\pi_0 = 1$ or $\pi_3 = 1$, then relatedness is undefined if either cooperators or cheats succeed in going to fixation. When calculating the average of relatedness (in a particular generation) over a number of replicates, we discarded those replicates in which fixation of cooperators or cheats had occurred.

SIMULATION ANALYSIS

We performed numerical simulations for each of the three dispersal regimes, providing a complete description of the population in terms of the frequency and distribution of cooperators and cheats over subpopulations and over time. We used our counts of CFU from the limited and budding dispersal regimes to calculate the absolute fitness (w) of cheating and cooperating strains in subpopulations founded by 0, 1, 2, or 3 cooperative colonies, which yielded the values of six fitness parameters. Stochastic sampling of clones to form each new subpopulation was implemented using a Mathematica random number generator, with the probability of success of the clone being determined by its fitness as in our empirical experiment (Fig. 1). We performed 10^5 replicates for each

of the three dispersal regimes and calculated the average values for the proportion of cooperators and relatedness as well as the range containing 95% of all simulated outcomes. Our experiment examined the extreme points of Taylor's (1992a,b) model, comparing selection for cooperation with the dispersal rates $d = 0$ and $d = 1$. To complement this, and assess the generality of our results, we also used the parameters estimated in our experiment to simulate the evolution of cooperation for intermediate levels of dispersal ($d = 0.1, 0.25, 0.5$).

Results

DISPERSAL REGIME AND THE EVOLUTION OF COOPERATION

We found that cooperation was favored by budding dispersal, but not by relatively limited dispersal (Fig. 2). The proportion of cooperators after 12 dispersal events differed significantly between the three dispersal regimes (ANOVA: $F_{2,15} = 5.14$, $P = 0.020$; randomization test: $P = 0.018$). Cooperation was equally disfavored with relatively limited and high dispersal, which justified the collapsing of these two factor levels (stepwise deletion: $F_{15,16} = 0.56$, $P = 0.47$). In contrast, budding dispersal favored the evolution of cooperation—the proportion of cooperators after 12 dispersal events was significantly higher with budding dispersal compared to individual (relatively limited and high) dispersal (Fig. 2; $F_{1,16} = 9.99$, $P = 0.006$; randomization test: $P = 0.007$). In addition, the variance in the proportion of cooperators varied significantly across dispersal regimes, with the variance across budding dispersal replicates being significantly higher than the variance across relatively limited and high dispersal replicates (F -test to compare two variances: $F_{5,11} = 5.26$, $P = 0.021$).

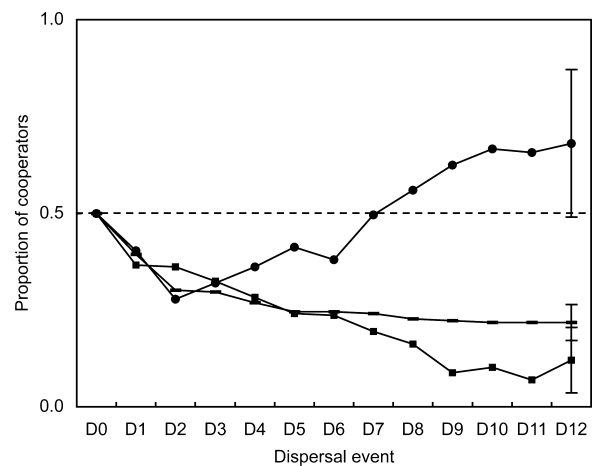


Figure 2. Dispersal regimes and the evolution of cooperation. The proportion of cooperators (mean \pm SE) after 12 dispersal events in *P. aeruginosa* populations with relatively limited dispersal ($d = 0$, bars), relatively high dispersal ($d = 1$, squares), and budding dispersal (circles).

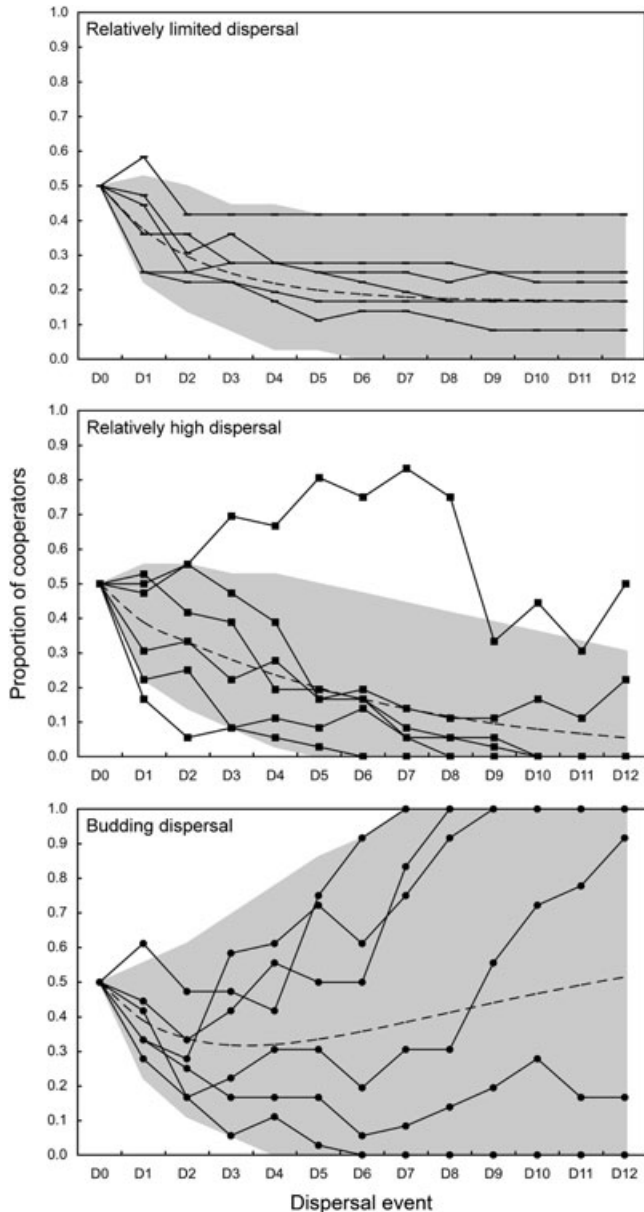


Figure 3. The evolution of cooperation: comparing experimental data with simulated data. Experimental data for relatively limited, relatively high, and budding dispersal are based on six replicates (solid lines) and simulated data are based on 10^5 replicates (mean indicated by dashed line). Shaded areas indicate the range containing 95% of all simulated outcomes.

Examining the relative success of cooperators and cheats in the different dispersal regimes, there was a good fit between our experimental data based on six replicates and the simulation data based on 10^5 replicates (Fig. 3). All experimental replicates of the limited and budding dispersal regimes, and five of the six high dispersal replicates lay within the 95% range of all simulated outcomes. Consistent with our experimental data our model predicts a higher variance in the proportion of cooperators in the budding

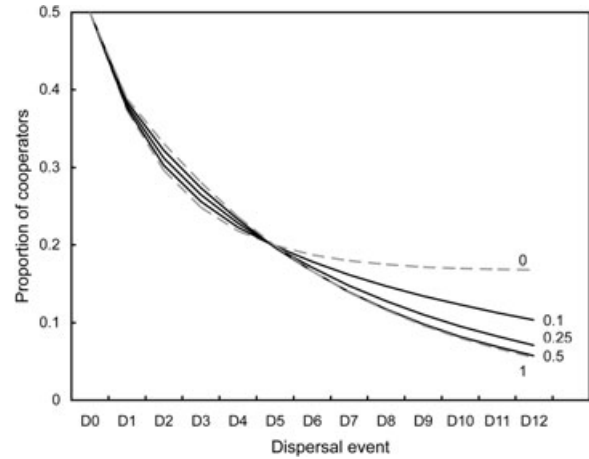


Figure 4. Intermediate dispersal rates and the evolution of cooperation. Simulated data (10^5 replicates) are based on fitness values from our experiment. Solid lines indicate intermediate dispersal rates ($d = 0.1, 0.25, 0.5$) between subpopulations, whereas gray dashed lines represent the two extremes from Taylor's (1992a,b) dispersal parameter range: $d = 0$, no dispersal between subpopulations, and $d = 1$, complete dispersal between subpopulations.

dispersal regime (variance = 0.171) than in the relatively limited (0.008) and high (0.011) dispersal regimes. In addition, simulations for intermediate levels of dispersal revealed that cooperation was disfavored across the entire range of d -values ($0 \leq d \leq 1$; Fig. 4).

DYNAMICS OF WITHIN SUBPOPULATION RELATEDNESS

Our experimental design allowed us to examine how different dispersal regimes influence the evolution of relatedness within subpopulations. Relatedness is a statistical measure (regression) of the genetic similarity of two individuals within a subpopulation, relative to the population average (see Methods). The relatedness that matters for social evolution (the r term in Hamilton's rule) is that for the trait being considered and not the genome-wide relatedness, nor the genealogical relationship between individuals (Hamilton 1964, 1975; Grafen 1985; Gardner et al. 2007). Normally, relatedness at different loci should be similar across the genome, which is why pedigrees or molecular markers are commonly used to measure r (Queller and Goodnight 1989). An advantage of microbes is that genetic knockout mutants can be generated, which allows r to be directly manipulated at the locus of interest, whereas the rest of the genome is initially held constant (i.e., pyoverdinin production in our case, West et al. 2006). If new mutations occurred at other loci during our experiment, the population structure imposed would lead to relatedness being the same at the mutated loci as at the pyoverdinin locus.

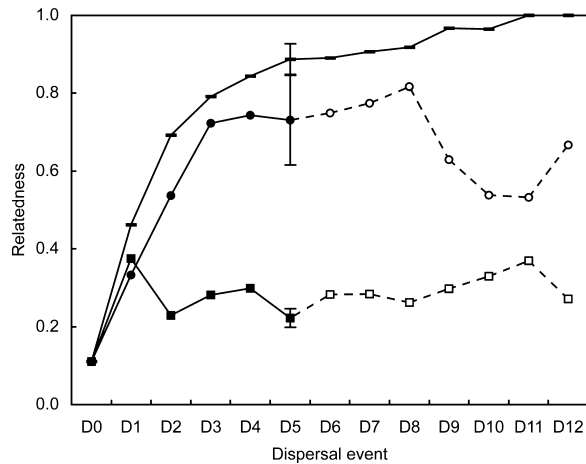


Figure 5. Dispersal regimes and the evolution of within subpopulation relatedness. Mean relatedness values (\pm SE) across six replicates are given for relatively limited dispersal (bars), relatively high dispersal (squares), and budding dispersal (circles). Open symbols connected by dotted lines indicate relatedness values based on a reduced number of replicates. This is because eight of 12 populations of the high and budding dispersal regime became fixed for either cooperators or cheats between D6 and D12, with relatedness being no longer defined for those populations. This leads to underestimated relatedness values for budding dispersal due to the populations with high relatedness being eliminated.

At the beginning of our experiments, relatedness was low in all populations. However, after just a few dispersal events, relatively limited and budding dispersal led to a high within-subpopulation relatedness (Fig. 5). For example, under relatively limited dispersal, relatedness reached an equilibrium value of $r = 1$ after 11 dispersal events. In contrast, relatedness did not increase in the relatively high dispersal regime (Fig. 5). Thus, relatedness after the fifth dispersal event differed significantly between the three dispersal regimes (ANOVA: $F_{2,15} = 28.15$, $P < 0.0001$). Stepwise deletion revealed that relatedness did not differ significantly between the relatively limited and budding dispersal regime, which justified the collapsing of these two factor levels ($F_{15,15} = 2.41$, $P = 0.142$). In contrast, relatedness was significantly higher in these dispersal regimes than in the relatively high dispersal regime ($F_{1,16} = 41.6$, $P < 0.0001$). We analyzed relatedness values from the fifth dispersal event because, in the following dispersal events, eight of 12 populations from the high and budding dispersal regime became fixed for either cooperators or cheats. This meant that relatedness was no longer defined for those populations, and the relatedness values for budding dispersal would have been underestimated, due to those populations with high relatedness being more likely to fix cooperators and hence being eliminated from the relatedness calculations.

Examining the evolution of relatedness, there was a close match between relatedness values from our simulations and the

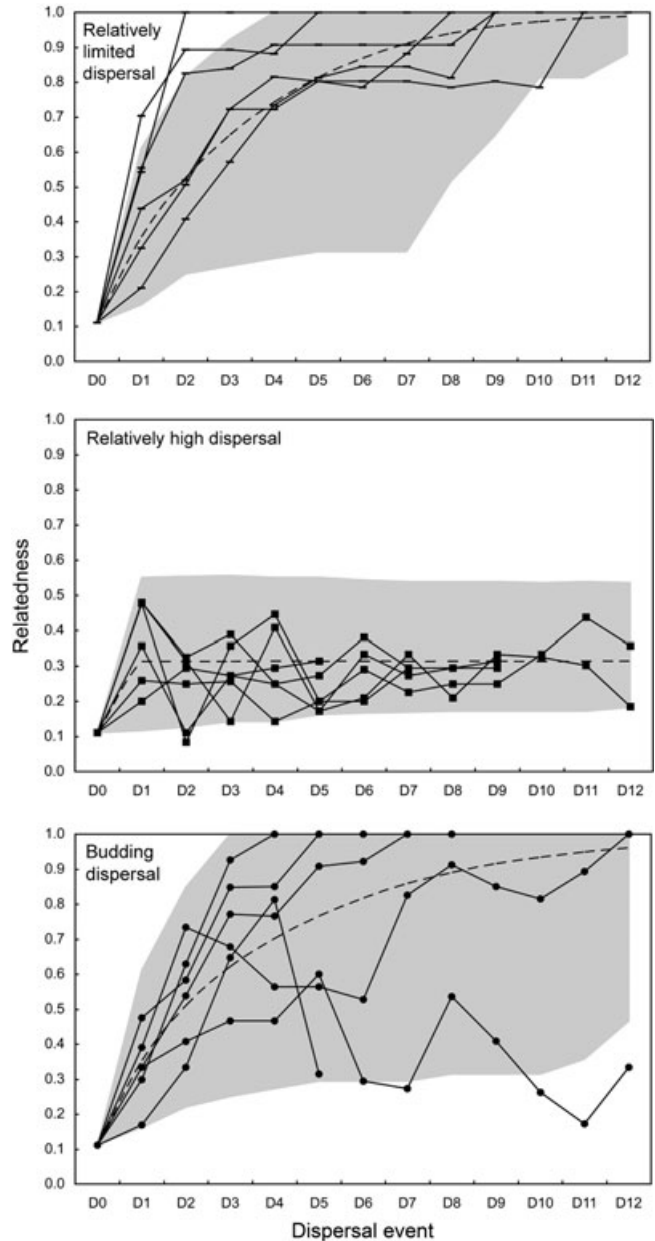


Figure 6. The evolution of relatedness: comparing experimental data with simulated data. Experimental data for relatively limited, relatively high, and budding dispersal are based on six replicates (solid lines) and simulated data are based on 10^5 replicates (mean indicated by dashed line). Shaded areas indicate the range containing 95% of all simulated outcomes. Eight of 12 populations of the high and budding dispersal mode became fixed for either cooperators or cheats between D6 and D12, with relatedness being no longer defined for those populations.

experimental values (Fig. 6). For all replicates of the relatively limited, relatively high, and budding dispersal regimes, the observed relatedness after the fifth dispersal event lay within the 95% range of all simulated outcomes.

Discussion

Limited dispersal has two potential influences on the evolution of cooperation and other social behaviors (Hamilton 1964, 1971, 1972, 1975; Taylor 1992a,b; Wilson et al. 1992). One effect is that interactions will tend to occur between relatives, which favors cooperation, and the other is that competition for resources will also tend to be among relatives, which opposes the evolution of cooperation (reviewed by West et al. 2002b). Our experimental results provide support for Taylor's (1992a,b) prediction that these effects cancel out, with the individual dispersal rate having no influence on the level of cooperation (Figs. 2 and 4). We also provide support for the prediction that a potential solution to this problem is if individuals disperse as groups, termed budding dispersal (Goodnight 1992; Gardner and West 2006; Lehmann et al. 2006). In that case, budding dispersal favors cooperation because it allows a high relatedness to be maintained, while reducing competition between relatives. Budding dispersal occurs in a range of cooperative species from unicellular organisms to humans (Haldane 1932; Peeters and Ito 2001; Clutton-Brock 2002; Pfeiffer and Bonhoeffer 2003; West et al. 2006; Sharp et al. 2008) where it could help favor cooperation.

LIMITED, HIGH, AND BUDDING DISPERSAL

As predicted by theory (Taylor 1992a,b; Wilson et al. 1992), the individual dispersal regime (relatively limited or relatively high) had no influence on the evolution of cooperation (Fig. 2). In the relatively limited dispersal regime ($d = 0$, no dispersal between subpopulations), the three colonies used to initiate each subpopulation come from the same subpopulation, leading to high relatedness (i.e., all subpopulations consisted of either cooperators or cheats after a few dispersal events). However, cooperation is predicted to be disfavored with this dispersal regime because limited dispersal also leads to competition between relatives within subpopulations (local competition). Put another way, although cooperative subpopulations are more productive, the benefits of cooperation cannot be exported. In contrast, in the relatively high dispersal regime ($d = 1$, complete dispersal between subpopulations), there is relatively low competition between relatives (global competition), and this means that more productive subpopulations contribute more to future generations. However, cooperation is disfavored in this regime because the three colonies used to initiate each subpopulation come from different subpopulations, leading to a relatively low relatedness (i.e., cooperators and cheats were often found in the same subpopulation with cheats successfully exploiting cooperators).

Taylor's (1992a,b) models predicted that the dispersal rate (d) has no influence on selection for cooperation. Although in broad agreement, our simulation analyses, using the parameters from our experiments, show two relatively minor differences compared to Taylor's model. First, the decrease in cooperator frequency is

slightly lower with smaller d -values (Fig. 4). The reason for this is that cooperators, although selectively disfavored, can become established by chance in some subpopulations, and are dislodged only after cheats have dispersed into those subpopulations. Consequently, the number of dispersal events required for extinction of cooperators increases as the dispersal rate decreases toward zero. We could demonstrate this effect in simulations based on 1000 replicates with 96 dispersal events, where cooperation became extinct in all replicates with $d > 0$, but with the time to extinction increasing with smaller d -values (for $d = 1$ and $d = 0.5$ extinction after 56 dispersal events, for $d = 0.25$ extinction after 71 dispersal events and for $d = 0.1$ extinction after 78 dispersal events). Second, with $d = 0$, our simulations suggest that some cooperators will be maintained in the population (equilibrium proportion of cooperators = 0.168). This is a result of our severe bottlenecks, where each subpopulation is founded by only three clones. This bottleneck allows cooperators, although selectively disfavored, to become fixed in some subpopulations due to random genetic drift. However, in the longer term and more natural conditions, new cheating mutants would emerge and spread with the effect of cooperation being also eliminated in populations with $d = 0$.

In contrast, cooperation is favored in the budding dispersal regime because it combines relatively high relatedness with relatively low competition between relatives (Gardner and West 2006). Relatedness is high because the three colonies used to initiate each subpopulation come from the same subpopulation. Competition between relatives is low because each subpopulation can contribute to more than one new subpopulation, with more productive subpopulations making a greater contribution to the next generation of subpopulations. There has been some disagreement in the theoretical literature over the consequences of budding dispersal—while some have suggested that it would select for cooperation (Goodnight 1992; Gardner and West 2006; Lehmann et al. 2006), others have suggested that it would select against it (Pollock 1983), or that it is not clear what should happen (Haldane 1932). Our experimental results provide clear empirical support for the prediction that budding dispersal favors cooperation. It should be noted that exactly the same predictions for the different dispersal regimes can be made from a kin or group selection perspective, as they are mathematically equivalent (Hamilton 1975; Grafen 1984; Wade 1985; Frank 1986; Queller 1992b; Gardner et al. 2007; Lehmann et al. 2007a; West et al. 2007b, 2008). We have used a kin selection approach because we find this easier to link to empirical parameters such as genetic relatedness, and because this is the approach that has been taken by the majority of theoretical work in this area (West et al. 2008).

How does our experiment relate to previous empirical work? Griffin et al.'s (2004) experiment manipulated relatedness and the amount of local competition between relatives independently, and

so although it showed that they both influenced selection for cooperation, it did not examine how their relative importance varied with the dispersal rate (see also Giron et al. 2004). Specifically, Griffin et al. (2004) kept dispersal high in all treatments by pooling subpopulations before composing the next generation. This was done to disentangle the effects of competition and dispersal, but precluded a direct test of Taylor's model. West et al.'s (2001) comparative study on fig wasps showed that, in the extreme case of completely local competition, relatedness had no influence on the level of fighting between males. Experimental studies have shown that lower dispersal rates favored lower parasite virulence (Kerr et al. 2006; Boots and Meador 2007). Virulence can be thought of as a social trait, with lower virulence a form of cooperative self-constraint, to share resources more prudently (Herre 1993; Frank 1996, 1998). Consequently, the optimal level of virulence is predicted to be influenced by the dispersal rate (Boots and Sasaki 1999), although it remains unclear why the effects of relatedness and competition in Kerr et al. (2006) and Boots and Meador (2007) did not cancel out, as in Taylor's (1992a,b) models. Limited dispersal has been shown to favor the production of antibiotic bacteriocins in bacteria (Chao and Levin 1981). A clearer prediction can be made in this case, as kin discrimination is also involved, and bacteriocin production is a spiteful behavior, and so favored rather than disfavored by local competition (Gardner and West 2004; Gardner et al. 2004). Finally, Wade (1976, 1977, 1982) and Wade and McCauley (1980) carried out a number of experiments on group selection using *Tribolium* beetles. Their aim was to examine how the pattern of dispersal influenced the rate at which populations responded to artificial selection on population size. Consequently, their experiments did not test how the pattern of dispersal influenced the relative success of cooperators and cheats, and hence the predictions made by Taylor's (1992a,b) model and its budding extension (Gardner et al. 2006). However, some of the dispersal patterns examined are very similar—for example, Wade's (1976) and Wade's and McCauley's (1982) treatments A and C are analogous to our budding and relatively limited dispersal regimes. It is noteworthy that almost all of the papers discussed above are laboratory experiments. Determining the importance of different dispersal regimes for the evolution of cooperation in natural populations will be the major task in the future (West et al. 2002b).

THE EVOLUTION OF RELATEDNESS AND THE DYNAMICS OF COOPERATION

Our experimental study allowed us to examine the short-term evolutionary dynamics of relatedness, and the resultant selection for or against cooperation. Previous theory in this area has examined the evolutionary end point, and not the evolutionary dynamics—i.e., models have examined when cooperation is favored, and not how it gets there (Taylor 1992a,b; Gardner and West 2006;

Lehmann et al. 2006). To determine how well the dynamics of our experimental results fit predictions, we extended the population genetic model of Gardner and West (2006) to predict the dynamics when starting with a mixture of cooperators and cheaters and using the fitness parameters from our experiment. We found a close match between the predicted values of our model and our experimental data (Figs. 3 and 6). Specifically, our model predicts an increase in the proportion of cooperators over time with budding dispersal, but a decrease with relatively limited and high dispersal (Fig. 3).

Our model can also explain why the budding dispersal regime shows an initial decrease in the proportion of cooperators (Fig. 2), as well as a high variation in overall cooperator success (Fig. 3C). In the budding dispersal regime, relatedness is initially low and therefore the proportion of cooperators is predicted to decline due to cheaters exploiting cooperators in mixed populations. However, relatedness increases over time as stochastic sampling effects lead to the formation of subpopulations that consist entirely of cooperators or cheats, which allows cooperators to spread. The high variance in cooperator success is due to the possibility that cooperation is driven to such a low frequency during the initial low-relatedness phase that it is lost from the population altogether, before it could be selected to go to fixation in the high relatedness phase. We would predict that such stochastic effects would become weaker with a larger number of subpopulations, as would be expected to occur in nature.

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