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Notes and Comments

Spite and the Scale of Competition in *Pseudomonas aeruginosa*

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abstract: Scale of competition has been shown to be an important factor in shaping the evolution of social interactions. Although many theoretical and experimental studies have examined its effect on altruistic cooperation, relatively little research effort has been focused on spiteful behaviors—actions that harm both the actor and the recipient. In this study, we expand on existing theory by investigating the importance of the global frequency of spiteful alleles, and we determine experimentally how the scale of competition affects selection for spite in the bacterial pathogen *Pseudomonas aeruginosa* under high and intermediate spatial relatedness. Consistent with our theoretical results, we found in our experiments that spiteful genotypes are more favored under local (rather than global) competition and intermediate (rather than high) spatial relatedness, conditions that have been shown to select against indiscriminate altruism.

Keywords: bacteriocin, greenbeard, harming, kin selection, relatedness, social evolution.

Introduction

Spiteful behaviors are those that reduce the fitness of both the actor and the recipient (Hamilton 1970; West et al. 2007) and have been identified in a range of organisms from bacteria to wasps (Hurst 1991; Foster et al. 2000; Gardner et al. 2004, 2007; Inglis et al. 2009). Understanding the selective forces that drive the evolution of spiteful behaviors is likely of particular relevance to disease-causing organisms, where spiteful interactions can have a profound effect on disease severity (Gardner et al. 2004; Buckling and Brockhurst 2008; Brown et al. 2009). Spiteful behaviors can potentially be favored when they harm individuals that have fewer genes in common with the actor than an average member of the competing population (negative "interaction" relatedness between the interacting individuals) and, hence, indirectly help individuals that have more genes in common with the actor (Hamilton 1970, 1972, 1996; Grafen 1985; Foster et al. 2001; Gardner and West 2004; Lehmann et al. 2006). This scenario requires that individuals can be discriminated on the basis of whether they have the same spiteful gene as the actor, either indirectly (e.g., through kin recognition) or directly, via a "greenbeard" mechanism (Hamilton 1970; Gardner and West 2004, 2010; West and Gardner 2010). Note that we make a distinction between interaction relatedness (formally, the relatedness coefficient in Hamilton's rule) and spatial relatedness. The former describes the genetic similarity of two individuals engaging in a particular type of social interaction (Grafen 2006), and the latter describes the tendency for the same genes to occur in close proximity rather than be spread evenly over the whole of the population (Rousset 2004). While this distinction is unimportant when social interactions are indiscriminate and are simply mediated by spatial proximity, interaction and spatial relatedness differ when individuals "choose" with whom to interact, as might be the case with kin recognition or greenbeards (Gardner and West 2010). However, in the latter case spatial relatedness determines which individuals have the potential to interact and, hence, influences the range of possible values of interaction relatedness.

Population viscosity (how far individuals disperse) can play an important role in interaction relatedness and, hence, the evolution of spite. Specifically, spite is maximally favored when the local frequency of genes for spite is intermediate and competition for resources is primarily local (Gardner and West 2004, 2010; Gardner et al. 2004;

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Inglis et al. 2009). Intermediate local frequencies of the spiteful allele (which can be associated with low to intermediate spatial relatedness but not high spatial relatedness) allow access to potential victims that do not carry the spite gene while ensuring that the benefit of harming victims is primarily realized by other carriers of the spite allele. Local competition favors spite because the third-party beneficiaries of spite are locals, which may carry the spite gene more often than individuals from the wider population. To put it another way, intermediate local frequencies and local competition mean that the targets of spite are less related to the actor with respect to the average member of the competing population (negative interaction relatedness). As competition becomes increasingly global, it is individuals from the wider population that stand to benefit most from resources freed up by spite, and they only infrequently carry the spite gene. However, this latter result may be largely driven by the assumption in these evolutionary models that the spiteful greenbeard is vanishingly rare and, therefore, exists by definition at low frequencies in the global (but not necessarily the local) population.

Here, we empirically address how the scale of competition affects the evolution of a spiteful behavior, anticompetitor toxin (bacteriocin) production, in test-tube populations of the opportunistically pathogenic bacterium *Pseudomonas aeruginosa*. We first expand on previous theoretical results to determine the importance of varying the global frequency of spiteful alleles, which in previous models was assumed to be very low. No empirical studies have explicitly determined the effect of the scale of competition on the evolution of spite, although the effect of low and high population viscosity, which simultaneously alters both spatial relatedness and the scale of competition, was recently investigated in the evolution of spiteful behaviors in nematode-associated *Xenorhabdus* bacteria (Vigneux et al. 2008). We independently manipulated spatial relatedness and the scale of competition to investigate their effects on the relative competitive ability of spiteful versus nonspiteful genotypes in an way analogous to that of a study by Griffin et al. (2004) investigating the evolution of indiscriminate altruism. We were, therefore, also able to compare how these variables differ in their effect on two key social traits of this organism, indiscriminate altruism and spite.

Material and Methods

Model

We constructed a mathematical model to investigate how the scale of competition and spatial relatedness affect the fitness of spiteful bacteriocin producers across a range of starting frequencies of these bacteriocin producers in the metapopulation. Previous models assumed the producers to be vanishingly rare, and it is currently unclear whether predictions regarding the effect of the scale of competition also hold at higher, experimentally tractable frequencies.

We considered an infinite population made up of patches within which all bacteriocin-mediated interactions occur. A proportion a of competitive interactions for resources also occurs within each patch, whereas a proportion $1 - a$ of competitive interactions for resources occurs across the whole metapopulation. As such, when a is high the fitness of a strain is relative to that of competitors with which they can potentially undergo bacteriocin-mediated interactions, whereas when a is low the fitness of a strain is relative to that of competitors with which they primarily will not be able to undergo bacteriocin-mediated interactions. We considered two strains of bacteria to be present in the population: a producer (P) strain that suffers a relative growth cost c in order to synthesize a toxin to which it is immune, and a nonproducer (N) strain that suffers a growth cost k in the presence of the toxin. The population frequency of producer cells is denoted p.

In the first variant of the model, we assumed that all patches are founded by a single bacterial cell (spatial relatedness = 1). Hence, there are two types of patches: P patches occur with frequency p, and N patches occur with frequency $1 - p$. The relative growth factor of producers is given by $g_P = 1 - c$, and the relative growth factor of nonproducers is given by $g_N = 1$, owing to nonproducers never coming into contact with the toxin. The population average growth factor is $g = pg_P + (1 - p)g_N$, and the fitness of the producer strain is given by its growth relative to that of its competitors:

$$w_P = \frac{g_P}{ag_P + (1 - a)g}. \tag{1}$$

Since $g_P < g$, we have $w_P \leq 1$ for all $a \leq 1$. Hence, the producer strain is never selectively favored when patches are clonal. Producer fitness w_P is a monotonically increasing function of scale of competition a: it increases from a minimum $w_P = g_P/g$ at $a = 0$ to a maximum $w_P = 1$ at $a = 1$.

In the second variant of the model, we assumed that each patch is founded by two independently chosen bacterial cells (spatial relatedness = 1/2). Hence, there are three types of patches to consider: PP patches occur with frequency p^2, PN patches occur with frequency $2p(1 - p)$, and NN patches occur with frequency $(1 - p)^2$. Growth of producers is $g_{P|PP} = g_{P|PN} = 1 - c$ in both PP and PN patches, whereas growth of nonproducers is $g_{N|PN} = 1 - k$ in PN patches and $g_{N|NN} = 1$ in NN patches. The population average growth factor is $g = p^2 g_{P|PP} + p(1 - p)(g_{P|PN} + g_{N|PN}) + (1 - p)^2 g_{N|NN}$, and the fitness of the producer strain is

$$w_P = p\frac{g_{P|PP}}{ag_{P|PP} + (1-a)g} + (1-p)\frac{g_{P|PN}}{a(g_{P|PP} + g_{P|PN})/2 + (1-a)g}. \quad (2)$$

Depending on parameter values, the fitness of the producer strain may be less than or greater than 1 and so can be favored or disfavored by selection (fig. 1). For ease of analysis, we will use a linear approximation for fitness (neglecting higher order terms of c and k):

$$w_P \approx 1 + (1-p)\left[a\frac{c+k}{2} + (1-a)pk - c\right], \quad (3)$$

which monotonically decreases with c and monotonically increases with k for all $0 < a,\ p < 1$—that is, a smaller growth cost of toxin production and a greater toxicity against sensitive bacteria increases the likelihood of the producer strain being favored by selection.

Fitness of the producer is a linear function of a that monotonically increases if $p < (c + k)/2k$ and that monotonically decreases if $p > (c + k)/2k$ (fig. 1). Hence, as long as $k > c$ (a necessary condition for the producer strain to be favored), then producer fitness increases as competition becomes more local at $p = 1/2$—that is, the condition imposed in the main experiment (see below). Note that $w_P \approx 1 + (1 - p)(pk - c)$ in the absence of local competition ($a = 0$) and that the condition for the producer strain to be favored here is $p > c/k$ (this is equivalent to the condition given in row 4 of table 2 in Gardner and West 2010). Hence, spiteful toxin production can be favored in the absence of local competition ($a = 0$). Note also that there is no effect of the scale of competition at the point $p = (c + k)/2k$. Assuming that bacteriocin-mediated killing is efficient ($k \gg c$), then this occurs at $p \approx 1/2$—that is, here we expect the scale of competition to have the smallest effect on fitness of producers when producers and nonproducers are approximately equally frequent in the population.

Finally, fitness of the producer is a quadratic function of p, and inspection of the second derivative (which is never positive) shows that this is a dome-shaped function; the maximum is always less than 1 but may be greater than or less than 0. Hence, over biologically feasible parameter space, fitness either is dome shaped or monotonically decreases with producer frequency.

In summary, consistent with previous work (Gardner and West 2004, 2010; Gardner et al. 2004; Inglis et al. 2009) the model demonstrates that bacteriocin producers are favored (1) when spatial relatedness is intermediate (50%) rather than high (100%), with spite never favored in the latter case (because interaction relatedness can never be negative in this scenario), and (2) when competition is local rather than global, when the starting frequency of the spiteful allele in the metapopulation is low. We additionally show that local compared with global competition also favors bacteriocin producers when producers are at frequencies of 50% or less within the metapopulation, although the effect of the scale of competition will decrease with increasing frequency within this range (fig. 1).

Bacterial Strains

Pseudomonas aeruginosa was used to study the effect that the scale of competition has on spiteful behaviors. *Pseudomonas aeruginosa* is an excellent study system for spiteful behaviors because it produces bacteriocins, proteinaceous anticompetitor toxins that kill susceptible bacteria (thus reducing the fitness of the recipient) and that are costly to produce given that cell lysis is required for their release (thus reducing the fitness of the actor; Nakayama et al. 2000; Michel-Briand and Baysse 2002; Cascales et al. 2007; Denayer et al. 2007). In this context, bacteriocin production can be considered a spiteful greenbeard because bacteriocin-producing bacteria are able to specifically harm social partners that do not share the same bacteriocin gene complex (allowing negative relatedness to occur; Gardner et al. 2004; Gardner and West 2010). We used strain PAO1 as a bacteriocin producer; serotype O:9 was used as the bacteriocin-sensitive competitor (Smith et al. 1992; Denayer et al. 2007; Inglis et al. 2009), which is susceptible to pyocin S2 produced by PAO1. This sensitivity can be readily confirmed using simple plate assays (Fyfe et al. 1984; Inglis et al. 2009). Bacteriocin production in *P. aeruginosa* involves only a few cells in the population at any one time that actively lyse to release the toxin. Although it is not clear whether lysis is required for the release of soluble pyocins, which are the focus of this study (it has been suggested that it is [Nakayama et al. 2000]), there is likely to be a metabolic cost to production (Michel-Briand and Baysse 2002).

PAO1 and O:9 have different genetic backgrounds, so differences in the fitness of these competing strains may be due to factors other than bacteriocin production and sensitivity. To control for this, we used an isogenic (to PAO1) pyocin S2 knockout mutant, PAO1150-2 (which is resistant to pyocin but no longer produces S2), to determine that the difference in fitness between PAO1 and O:9 is primarily due to pyocin production and sensitivity (Jacobs et al. 2003; Inglis et al. 2009; fig. 2).

Competition Experiments

Short-term competition experiments between the bacteriocin-producing, bacteriocin-nonproducing, and bacte-

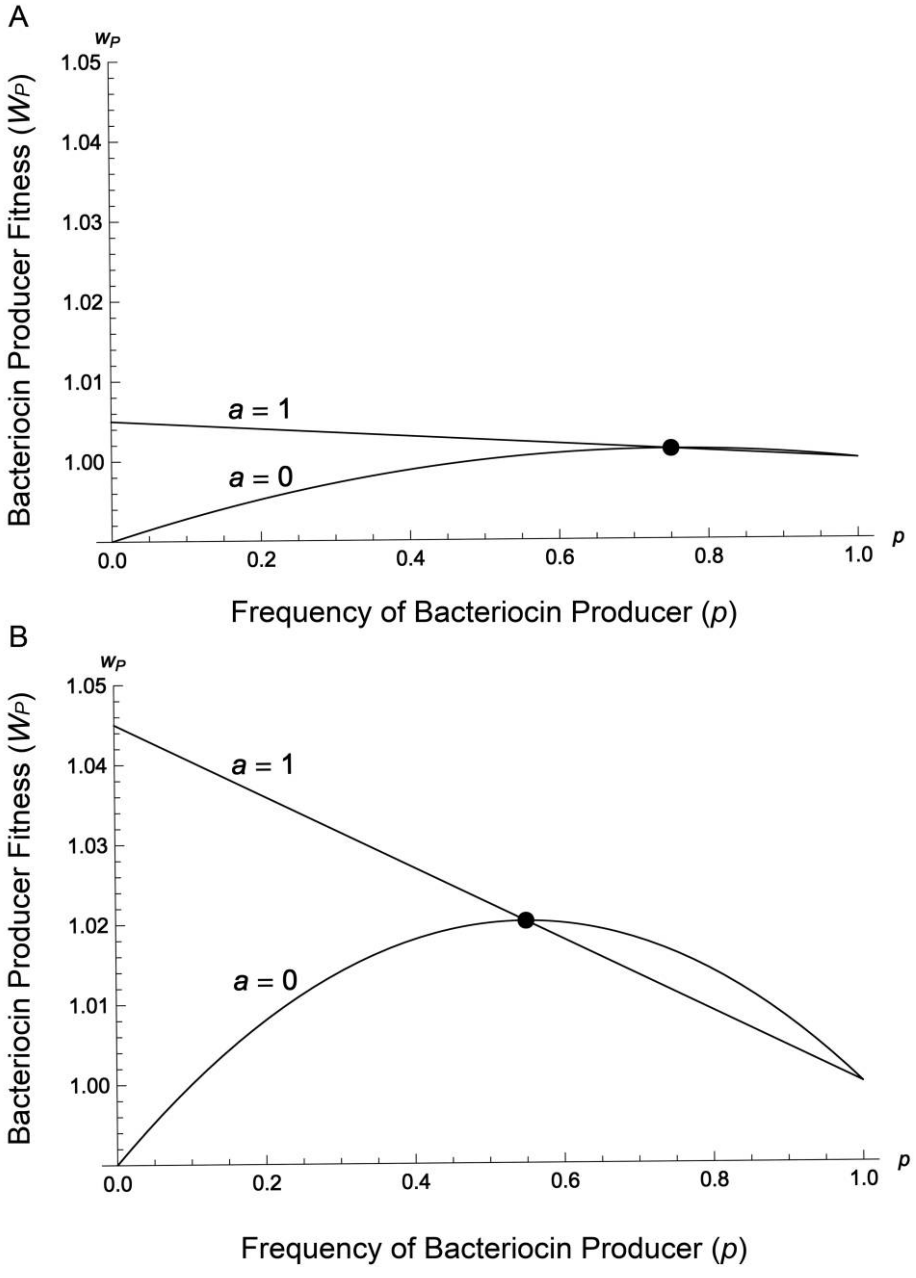

Figure 1: Model results. Shown is the fitness of a bacteriocin producer as a function of global starting frequency in the metapopulation under conditions of entirely local ($a = 1$) or global ($a = 0$) competition. Two individuals are present in each patch. *A*, $c = 0.01$ (where c is cost to actor), $k = 0.02$ (where k is cost to recipient; hence, $k/c = 2$). *B*, $c = 0.01$, $k = 0.10$ (hence, $k/c = 10$). Note that there is no effect of scale of competition (a) on bacteriocin producer fitness (w_P) at the point $p = (c + k)/2k$ (this value is marked by a dot on both graphs). Assuming extremely efficient bacteriocin action ($k \gg c$), then this occurs at $p \approx 0.5$.

riocin-sensitive strains were conducted at a range of starting frequencies to determine the generality of our theoretical results and confirm that the observed differences in fitness were in fact due to pyocin production and sensitivity (fig. 3). Overnight cultures of each strain were grown with shaking at 0.65 *g* at 37°C for 18 h and then diluted to an optical density of 1.8 measured at 600 nm to ensure similar numbers of bacteria per milliliter. These cultures were subsequently grown on agar plates to determine the number of bacteria present, with colony-form-

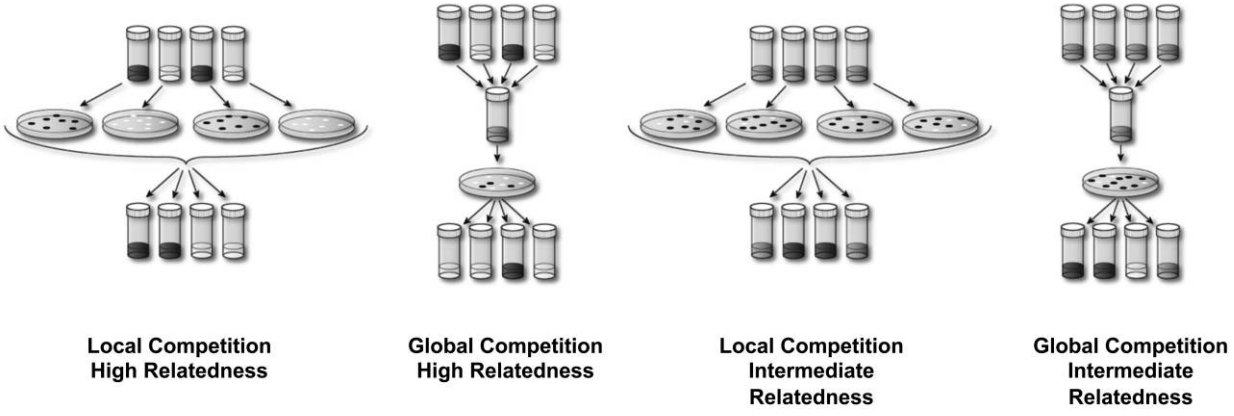

Figure 2: Experimental design. Spatial relatedness was varied among interacting individuals by inoculating each subpopulation with either a single bacterial clone (high genetic relatedness) or two bacterial clones (intermediate genetic relatedness). The scale of competition was manipulated either by mixing all of the subpopulations from a treatment before plating (relatively global competition) or by allowing each subpopulation in a treatment to provide equal numbers of colonies to the next generation (local competition). Here, black represents the bacteriocin-producing strain (PAO1), white represents the sensitive strain (O:9), and gray represents a mix of the two.

ing units as an approximate measure. Thirty-milliliter glass universals containing 6 mL of King's medium B (KB) broth were inoculated with a total of 10^4 cells with different starting frequencies of the individual strains. PAO1 (bacteriocin producer) and O:9 (bacteriocin sensitive) were competed against each other at starting frequencies of 99%, 90%, 50%, 10%, 1%, and 0.1%. The isogenic knockout mutant PAO1150-2 (bacteriocin nonproducer) was competed directly against O:9 at a frequency of 50% (i.e., grown in the same tube) and was also competed indirectly against O:9 (i.e., grown in separate tubes). Cultures were propagated in a shaking incubator at 0.65 *g* at 37°C and sampled after 96 h, allowing time for the effect of the bacteriocin to be observed. We calculated the growth of both the producer and nonproducer relative to that of the sensitive strain at the different starting frequencies. This was done by plating the various treatments on KB agar plates and counting the number of colony-forming units for each strain. All strains were easily distinguishable from one another because of unique colony morphology and size. At the more extreme frequencies, antibiotic plates were required to give better resolution of colony counts, and this was possible because of the different antibiotic resistance profiles of the assorted strains (PAO1, resistant to 1,250 μg/mL streptomycin; O:9, resistant to 312.5 μg/mL rifampicin; PAO1150-2, resistant to 312.5 μg/mL tetracycline). Relative fitness (w) was used to estimate at what frequency bacteriocin production is favored in PAO1 relative to PAO1150-2 using the common competitor O:9, where $w = m_j/m_i$ and m refers to ln (final density/starting density) of strain j (in this case either PAO1 or PAO1150-2) and strain i (O:9; Lenski et al. 1991). Assays at each frequency were replicated six times.

Metapopulation Experiment

We wanted to test the qualitative predictions from our model that intermediate versus high spatial relatedness and local versus relatively global competition favor bacteriocin producers. We used a single global starting frequency of the producer (50%), both for experimental tractability and for the sake of parsimony: our model suggests that the effects of the scale of competition are predicted to decrease up to 50%. Overnight cultures of PAO1 and O:9 (the producer and sensitive bacteria, respectively) were grown in 30-mL glass universals containing 6 mL of KB broth with shaking at 0.65 *g* at 37°C for 18 h and were subsequently diluted and then plated onto KB agar petri dishes. These plates were then incubated for an additional 18 h at 37°C, and the bacterial colonies from the agar plates were used to start the experiment. We independently manipulated spatial relatedness and the scale of competition in a fully factorial design (fig. 2). Replicates contained one population divided into six subpopulations. Each subpopulation was grown in a tube of KB broth. All treatments were inoculated with bacterial cores containing 10^6 cells of bacteria for both the producer and sensitive strains, obtained by stabbing colonies from the agar plates with a 1-mL sterile pipette. Bacterial cores were also serially diluted and plated to confirm that the densities were in fact the same (data not shown). To achieve high spatial relatedness, we initiated each subpopulation (12 in total) with a single bacterial clone: in the first generation, half of the

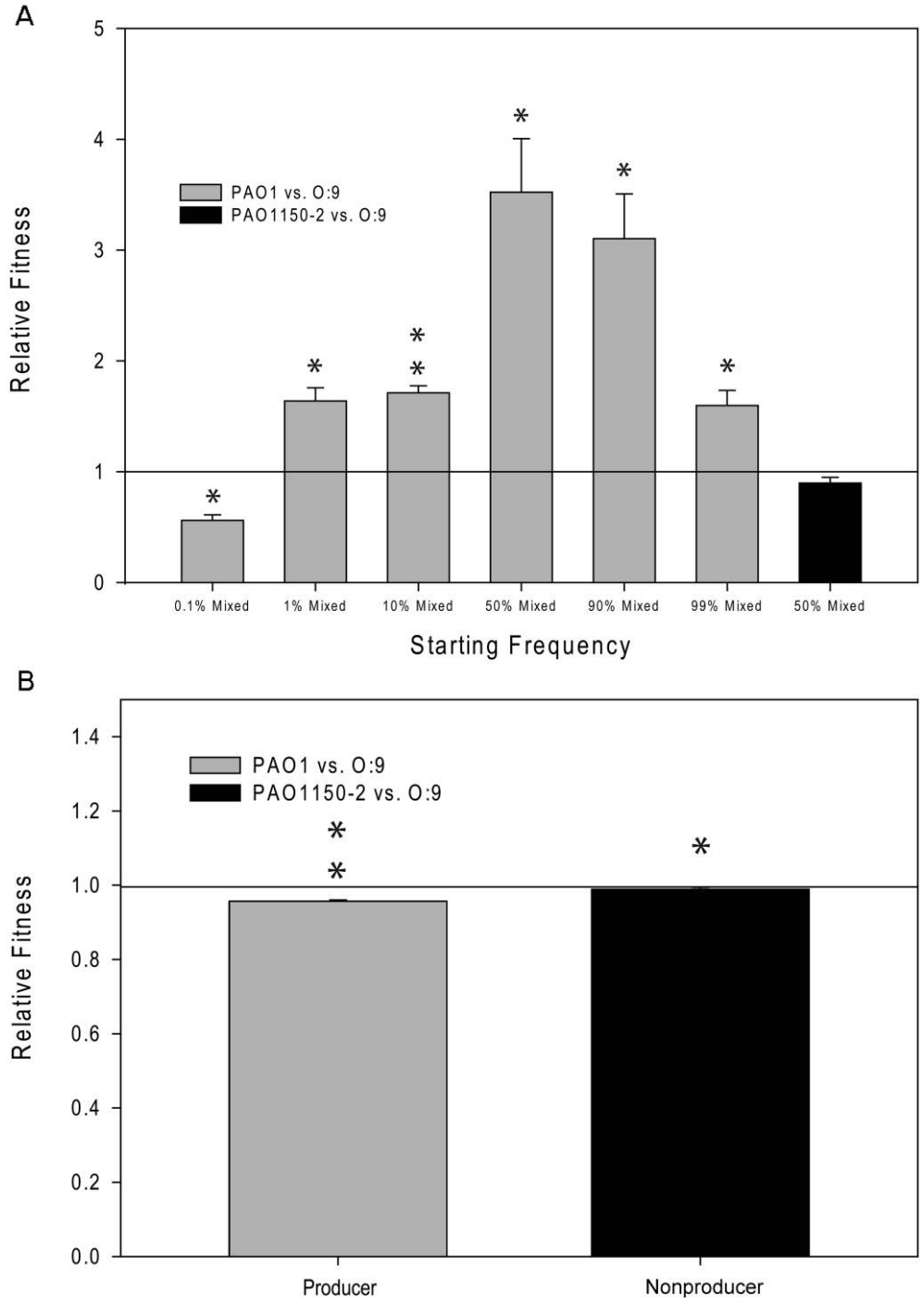

Figure 3: *A*, Invasion of a bacteriocin producer (PAO1) and an isogenic nonproducing mutant (PAO1150-2, which does not produce pyocin S2) under a range of starting frequencies when in direct competition with the sensitive strain (O:9). The producers are able to invade when starting at 1% of the population but show the greatest relative fitness at 50 : 50 frequencies. When this is compared with the nonproducer (*black*), there is a large difference in fitness attributable to the pyocin production. *B*, Relative fitness of the producer and nonproducer when in indirect competition with the sensitive strain. This illustrates that when grown as a monoculture the sensitive strain has a fitness advantage compared with the producer but not with the nonproducer. Error bars show SEMs in both graphs. Asterisks indicate *P* values for two-tailed *t*-tests analyzing the difference between the mean of each treatment from 1 after a sequential Bonferroni procedure had been applied (Holm 1979; *two asterisks*, $P < .01$; *one asterisk*, $P < .05$; *no asterisks*, $P > .05$).

subpopulations in a treatment were initiated with the producer strain, and the other half were initiated with the sensitive strain, giving an overall 1 : 1 ratio of producing to sensitive bacteria (fig. 2). To contrast this, we imposed intermediate spatial relatedness by initiating each subpopulation with two genetically different bacterial clones: in the first generation, all of the subpopulations were initiated with a 1 : 1 mix of producing and sensitive individuals. Cultures were grown for 10 h in a 37°C orbital incubator, with shaking at 0.65 *g*.

We imposed relatively global competition by mixing the cultures from all of the subpopulations in a treatment before plating, incubating plates for 18 h at 37°C, and then transferring random colonies (one or two for high and intermediate spatial relatedness treatments, respectively) from this single plate to initiate new subpopulations (fig. 2). This procedure allows productivity in a tube to determine the genetic contributions to subsequent generations within the whole metapopulation. In contrast, we imposed local competition by plating all tubes separately and choosing random colonies from randomly selected plates to inoculate tubes, removing any advantage from being in a productive tube (Griffin et al. 2004). Note that colony cores were sampled using a 1-mL pipette tip, resulting in similar numbers of bacteria being sampled from all colonies. This selection procedure was repeated for five transfers. For every round of selection we scored the frequencies (on the basis of their distinct colony morphologies) of the producer and sensitive bacteria growing on the agar plates, and the relative proportions were inoculated into the next set of tubes. We used the proportion of producer bacteria inoculated into the next generation as the response variable in our analyses and in figure 4. The whole experiment was replicated six times.

Analyses

For the main experiment, we determined how the proportion of bacteriocin producers was affected by spatial relatedness and the scale of competition in a general linear mixed model. The proportion of bacteriocin producers was arcsine-square-root transformed to meet with model assumptions (normality and homogeneity of error), with spatial relatedness, scale of competition (both two-level factors), time (six-level factor), all two-way interactions, and strain replicate (random effect) fitted as explanatory variables while accounting for autocorrelation. To analyze the short-term competition data, *t*-tests with a sequential Bonferroni correction (Holm 1979) were used. All analyses were conducted in R software (ver. 2.9.2).

Results

We conducted short-term experiments to establish qualitative consistency between our experimental and theoretical work. We first identified a cost of bacteriocin production by comparing the growth rate of our bacteriocin-producing (pyocin S2) strain with that of an isogenic mutant (PAO1150-2) that does not produce pyocin S2 when grown in isolation (fig. 3). We also demonstrated that in isolation the relative growth rate of the sensitive strain (which does not have the same genetic background as the producer) is greater than that of the producer but does not differ from that of the nonproducer (fig. 3*B*). This suggests that the different growth rates of the producer and sensitive strains can be explained to some extent by pyocin production. Moreover, when we competed the nonproducer and sensitive strains in the same tube at equal starting densities, we found that the sensitive strain had no significant growth rate advantage (fig. 3*B*). In contrast, the producer showed a massive fitness advantage against the sensitive strain under these conditions (fig. 3*A*). These results confirm that pyocin production confers an absolute growth rate cost but can confer a large fitness advantage when in direct competition with sensitive strains, as assumed in our theoretical work.

We next investigated how the relative fitness of the producer when in direct competition with the sensitive strain varied with starting frequency (fig. 3*A*), as we have previously shown in another experiment (Inglis et al. 2009). Consistent with this previous work (Inglis et al. 2009), we found a large fitness advantage of the producer at all but very low starting frequencies; in this latter case, the relatively small reduction in competitor frequency (through the killing action of the pyocin) is outweighed by the cost of pyocin production. Given the qualitative consistency of results at all but very low frequencies, we chose a single global starting frequency of the spiteful lineage (50%) for the larger metapopulation experiment. This frequency was chosen both for experimental ease (similar starting frequencies allow more accurate estimates of changes in frequency) and for the sake of parsimony: our theoretical results demonstrated that a 50% frequency should minimize the effects of the scale of competition, compared with lower frequencies.

In the metapopulation experiment, we independently manipulated the scale of competition (local and global) and spatial relatedness (high or intermediate) with the producer and sensitive strains at 50% starting frequencies. Our theoretical results suggested that both intermediate spatial relatedness and local competition should favor the producer. There was no significant interaction between the scale of competition and spatial relatedness ($F_{1,117} = 2.36$, $P > .14$; this interaction term was subsequently discarded from the model; fig. 4), although there was a trend

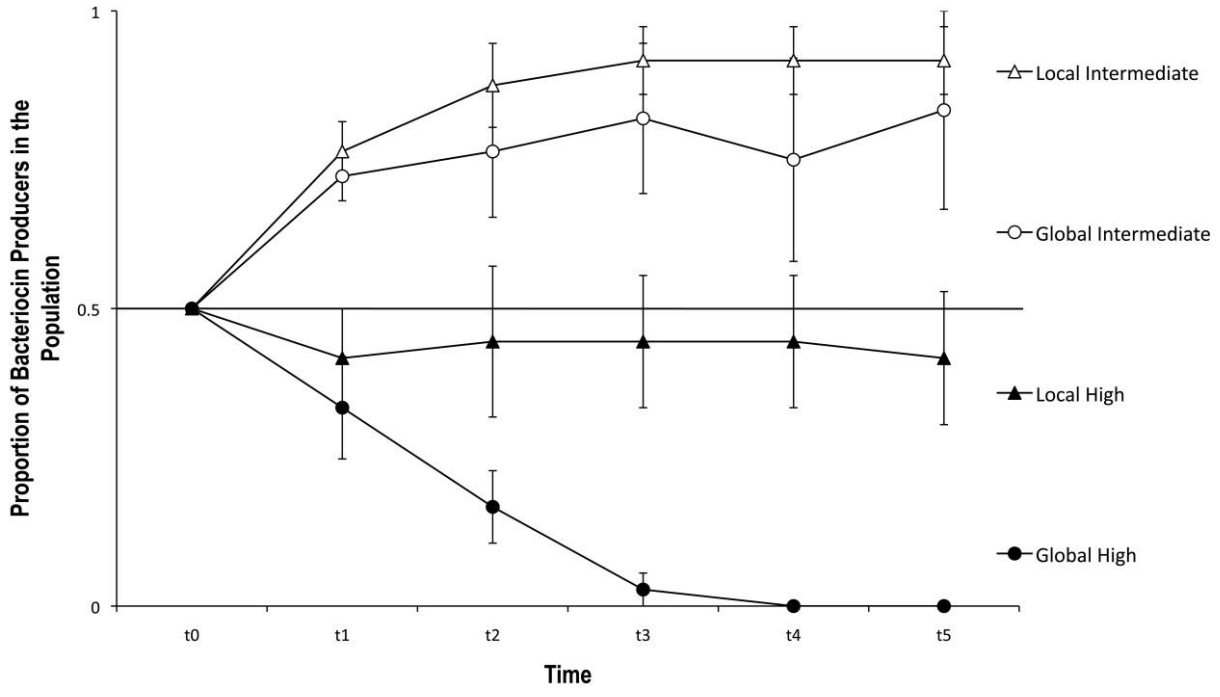

Figure 4: Change in frequency of bacteriocin producers in response to the scale of competition and spatial relatedness. The graph illustrates the effect of the scale of competition and local frequency in our experimental system. Points on the graph indicate the mean proportion of bacteriocin producers in the metapopulations within individual treatments. Error bars show SEMs. Bacteriocin producers are favored under conditions of low local frequency for both global and local scales of competition.

toward the scale of competition having a more pronounced effect when subpopulations were initiated with single clones (high spatial relatedness). However, the mean success of the producer was greatest under local competition (main effect of scale of competition: $F_{1,117} = 6.08$, $P < .015$) and under intermediate spatial relatedness (main effect of relatedness: $F_{1,117} = 43.16$, $P < .0001$; fig. 4). This is because the producer under these conditions will frequently encounter sensitive competitors (a necessary condition for negative interaction relatedness), and its reduced intrinsic growth rate relative to that of the sensitive strain does not have fitness consequences. In contrast, under conditions of high relatedness and global competition the sensitive strain went to fixation within all the experimental populations. Under these conditions the sensitive strain does not encounter the producer (interaction relatedness is not negative); hence, the sensitive strain's higher intrinsic growth rate allows it to dominate the global population. Note that there was not a significant change from the 50% starting frequency of the nonspiteful genotype under high spatial relatedness and local competition because there was no opportunity for fitness differences between strains in this context. In this case, the treatment acts as a control to illustrate that there are no underlying biases in this type of selection regime. These findings are entirely consistent with our theoretical predictions.

Discussion

Our results provide a clear experimental demonstration of how population viscosity influences the evolution of spiteful behaviors in the form of both scale of competition and spatial relatedness. Specifically, both intermediate spatial relatedness and local competition increased the fitness of bacteriocin-producing *Pseudomonas aeruginosa* relative to that of nonproducing sensitive *P. aeruginosa*. The producing genotype had a fitness advantage when spatial relatedness was intermediate under both local and global competition and a disadvantage under conditions of high spatial relatedness and global competition. Consistent with our theoretical work, the effect of the scale of competition occurred at the high producer starting frequencies used in the experiment (50%); previous theory considered only low starting frequencies. A previous study by Griffin et al. (2004) that used a virtually identical experimental design found that indiscriminate cooperative behaviors (the production of iron-chelating siderophores in bacteria) were favored under conditions of global competition and high

spatial relatedness and were disfavored under conditions of local competition and intermediate spatial relatedness. This suggests that simultaneous expression of these traits (likely to be the case in many bacteria) may have antagonistic effects on overall fitness, given that conditions that favor cooperative behaviors disfavor spiteful behaviors and vice versa.

The scale of competition is likely of particular importance in microbes because they often grow attached to surfaces with limited movement and, hence, relatively local growth and competition, but they can also disperse over relatively large distances (Velicer 2003). Overall, the scale of competition is likely to vary continuously across microbial species depending on dispersal rates, and in the context of bacterial pathogens this variation will occur as a result of within-host growth (local) and transmission to new hosts (relatively global).

Both the scale of competition and spatial relatedness can be affected by dispersal, with often-opposing effects on selection for social behaviors (Hamiltion and May 1977; Crespi and Taylor 1990; Koenig et al. 1992; Perrin and Lehmann 2001). Specifically, dispersal decreases spatial relatedness (favoring spite) but makes competition more global (disfavoring spite). However, unlike social acts that do not involve greenbeard recognition, bacteriocin production (and, hence, negative interaction relatedness) can theoretically be favored under a range of local frequencies (>0% and <100%) and under all scales of competition except global competition in infinite populations (Lehmann et al. 2009). As populations are, of course, never infinite, this implies that dispersal should generally favor spiteful acts (i.e., the only dispersal condition that will select against spite is when there is no dispersal). This view is consistent with the results of a previous study using a bacteria-nematode system that reported greater interference competition (bacteriocin production) under conditions of high dispersal compared with low dispersal (Vigneux et al. 2008).

In summary, our study provides the first empirical support that local competition for resources favors the evolution of spiteful behaviors. Moreover, we show both empirically and theoretically that this is the case even when spiteful genotypes are at quite high starting frequencies (up to 50% or higher) in the global population and not just when the spiteful genotypes are vanishingly rare, as in previous theoretical work (Gardner and West 2004, 2010; Gardner et al. 2004). However, while scale of competition is clearly important for the evolution of spite, our results suggest that the local frequency of the spiteful allele will frequently play a more important role. Spite is likely to be an important social interaction in bacteria in general, given that nearly all bacterial isolates have been found to produce bacteriocins (Riley and Wertz 2002), and in a clinical setting more specifically, given that many *P. aeruginosa* clinical isolates have been found to produce pyocins (Govan 1986). Understanding microbial social behaviors may explain how infections change in their virulence, which may lead to innovative therapies.

Acknowledgments

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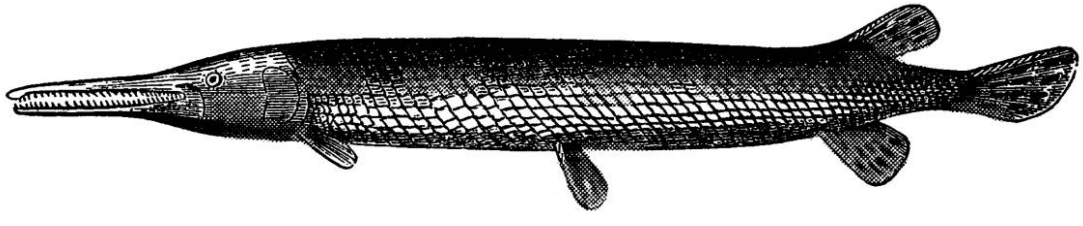

Gar pike (*Lepidosteus osseus*). From "Notes on Fresh-Water Fishes of New Jersey" by Charles C. Abbott (*American Naturalist*, 1870, 4:99–117).