Selection of spiteful behaviors under different interaction scales

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ABSTRACT

Spiteful behaviors are social interactions where actors pay fitness costs to harm recipients. For them to evolve, it is necessary that harming population members represents a reliable strategy to benefit closely related individuals. This is challenging to achieve in natural populations and it has long been debated what conditions could lead to positive selection of spite. It is clear that spatial scales of interaction play an important role, but most theoretical work has not modelled these scales explicitly. To study conditions for the selection of spite, we developed an agent-based simulation model inspired by bacteriocin production, where these spatial scales are explicitly included as tunable parameters. We provide broad parameter sweeps, varying the costs of production, initial producer frequency, population resistance and field density over many combinations of spatial interactions. Our simulation results reveal toxin producers benefit from local competition, large spite ranges and limited dispersal. The selection of producers is also highly sensitive to the producer frequency, baseline toxin resistance and field density. Additionally, we briefly review and discuss a selection of relevant toxin production models and experiments. Our model provides readily interpretable results for varying spatial scales and is a contribution to the understanding of allelopathic spite selection.

1 INTRODUCTION

In nature, it is common to observe social interactions between and within animal, plant and microorganism species. These behaviors represent important evolutionary driving forces and are themselves subject to natural selection. They can be classified by the direct fitness effects that incur on the actor and recipient of the social interaction: cooperation when both parties benefit (+/+); selfishness when the actor benefits at the expense of the recipient (+/-), altruism when the actor pays a cost to benefit the recipient (-/+); and spite to harm it (-/-) [14, 45].

Altruism and spite were initially puzzling in social evolutionary theory, since natural selection at the level of the individual should eliminate behaviors that are individually costly. However, when considering the selection at the level of genes and the traits encoded by them, what matters is the inclusive fitness of an individual, that is, the sum of its fitness and of all other individuals weighted by genetic relatedness at the relevant loci [14, 45].

In his seminal work, Hamilton realized that individually costly behaviors could evolve if the fitness benefit B towards individuals with genetic relatedness R outweigh their positive cost C, summarized in the relationship $RB > C$ [23, 25]. This came to be known as Hamilton's rule, and is a condition for positive selection of altruistic and spiteful behaviors. Selection of altruism requires sufficiently positive benefits towards positively related individuals, while spite requires negative benefits (or detriments) towards negatively related ones. Spite is often interpreted as a form of indirect altruism, where closely related individuals benefit from the harm caused to others. When this third party benefit is made explicit, this is called Wilsonian spite, while describing the interaction only with negative relatedness is termed Hamiltonian spite [14].

The concept of negative relatedness is not immediately intuitive, but it can be interpreted geometrically as individuals being less related to others than to the population average [12, 14, 19]. Relatedness to the whole population must add to zero, as randomly selected individuals should have zero relatedness on average. For large population sizes, only small values for negative relatedness are possible, but this can be augmented through kin discrimination mechanisms that allow individuals to choose interaction partners or modulate the strength of social interaction [39].

Independently, Price also showed that selection for spite and altruism was theoretically possible. He realized that using covariances and regression coefficients between traits and fitness w is a general way to mathematically describe natural selection. This was formalized in the Price equation $\Delta\overline{\phi} = \mathrm{Cov}(\phi, w) + \overline{w\Delta\phi}$ which describes mean changes of a trait ϕ in a population over a generation [36]. Indeed, different variants of Hamilton's rule can be derived using Price's formalism and model-specific measures for cost, benefit and relatedness based on covariances [19, 32].

An additional way to conceptualize natural selection of behaviors is through multilevel selection theory, decomposing between- and within-group selection [32]. Natural populations are frequently structured as meta-populations, consisting of semi-isolated groups with variance in genotype/phenotype frequencies and occasional exchange of genetic material. When behaviors have a positive individual fitness cost but are still positively selected, a Simpson's paradox is created [7]. For example, in bacterial public-good production with cooperators and non-producing cheaters, expressing the behavior is associated with a lower fitness within each group as it is metabolically costly. This is advantageous to cheaters and may lead to a decrease in the frequency of producers within groups.

Despite this, if public-good production leads to a sufficiently higher growth rate, groups containing producers have an advantage and producers can increase in frequency in the meta-population. This exemplifies that selection for behaviors at different levels can have conflicting and counteracting effects.

Spite was relatively neglected in early social evolutionary theory as it was considered that the conditions that would allow for spite selection were too restrictive or non-existent [14, 24, 45]. Even Hamilton initially dismissed it as a biologically relevant interaction, as it seemed to be always counter-selected or applicable to populations in catastrophic decline with small population sizes [14, 24]. In evolutionary models, global competition was often assumed, which importantly decreases any selective advantage for spite. Theory also predicted spiteful behaviors would be selected only if fitness costs are minimal and targeting of negatively related individuals is reliable. However, in natural populations there is a tendency to interact with kin (higher relatedness) due to physical proximity after reproduction and limited dispersal (population viscosity) [37, 42]. Accurate kin discrimination can help, but mechanisms for this are biologically rare [25]. An additional concern was that many behaviors originally labeled as spiteful were later reinterpreted as selfish, as they lead to fitness benefits to the actor in the long run [12, 45]. Examples of this are infanticide, egg cannibalism and attacks to neighboring nests, which eventually free up resources for the actor. Collectively, these issues raised doubts whether spite could be positively selected.

Nevertheless, spiteful behaviors are indeed observed in nature. In animals, they are more easily identified in insect communities with eusociality, where there is a division of reproductive roles between queens and workers [12, 14, 45]. A classic example is found in the red fire ant *Solenopsis invicta* that carries a green beard allele *b* at the locus Gp-9 [11, 29]. Green beards are phenotypic markers that allow kin discrimination, such that ant workers with the green beard allele (Bb) can identify and kill queens that are not carriers (BB). Although this could in principle eliminate the B allele, recessive homozygosity for Gp-9 (bb) is not viable, which maintains a competitive balance between Bb and BB queens. In this case, kin discrimination through a genetic marker is used to direct spite towards negatively related queens and fitness costs are limited as fire ant workers are sterile [12].

Another prime example of spite in nature is allelopathy, the chemical manipulation of environments for interference competition [3]. Importantly, allelopathic interactions may be either selfish or spiteful, depending on the overall fitness cost balance to individual producers. Allelopathy is widespread in microbial communities, leading to a chemical warfare with an enormous diversity of compounds [20, 31, 40]. Bacteriocins are protein toxins found in almost all known bacteria with up to hundreds of different toxins within a species [20]. Interestingly, many bacteriocins have a relatively narrow phylogenetic killing spectrum, targeting mostly closely related bacteria through specific protein receptors [40]. Common mechanisms of action of these toxins are metabolism interference through enzymatic inhibition, membrane pore formation or nuclease activity against DNA or RNA. There is frequently a genetic linkage between the bacteriocin and immunity genes to protect other carriers. In this case, bacteriocin gene clusters can be interpreted as a green beard trait, allowing bacteria to preferentially target non-carriers [2, 45].

The most extensively studied family of bacteriocins are colicins produced by *Escherichia coli*. They are large proteins that require lysis of the producing cell for toxin release, with many different types and mechanisms of action. Usually, they create pores in the membrane or degrade nucleic acids, and recognition of target cells is done through specific protein receptors. Colicin gene clusters are encoded in plasmids and generally contain genes for toxin production, immunity through toxin inactivation, and cell lysis [20, 38, 40].

Experimental and theoretical work to understand conditions for spite selection has been performed with a variety of approaches. [4, 6, 10, 13, 15–17, 21, 27, 28, 30, 44]. Although it has become clear that spatial structure and the scales of interaction between individuals play an important role in the selection of spiteful traits, most available work has not modeled these spatial scales explicitly. Also, while some conditions that benefit spite can be identified, it is not always clear they can be sustained in dynamical models. For example, while large population sizes make negative relatedness tend to zero, it has been argued that higher values of negative relatedness can be achieved if competition ranges are small, so that few individuals interact with each other [14]. However, if space is explicitly included in the model, with limited dispersion there is a tendency for spontaneous assortment increasing relatedness, as in natural populations [37, 42]. At the same time, viscosity promotes local competition, so benefits from competition reduction follow spatial relationships and tend to remain close to toxin producers. Therefore, the interplay between the range of spiteful interactions, the size of the competition arena and dispersal of individuals importantly affects when Hamilton's rule can be satisfied.

To study what combinations of spatial scales promote the selection of spite, we developed a minimal model of allelopathic spite evolution. We explicitly model the spite range, the size of the social competition arena and the dispersion of individuals and analyze how this interacts with other model parameters such as field density, spite cost, toxin resistance and producer frequency. Additionally, we provide a brief review of a selection of existing theoretical and experimental work.

2 METHODS

2.1 Model Description

To study conditions for spite evolution and selection we developed an agent-based simulation model. Although it was biologically inspired by bacteriocins, it can be adapted or interpreted to model other types of spiteful behaviors. The model was written as a python 3.8 script, and its initial implementation was done as part of Silvia Espada Burriel's master internship (VU 2018-2019). Parameters and dynamics for the model are outlined in the sections below.

Individuals are placed in a 2-dimensional square grid with L^2 points of size Δx. The field has periodic boundaries, wrapping

around and connecting the edges of the lattice. Individuals can reproduce, disperse and die during each time step. Dispersion steps are introduced in between both growth and death events to better converge to the intended continuous time dynamics. We assume the effect of spite is to increase the death rate of interacting recipients while incurring growth rate costs to actors. The degree to which individuals experience spite and resource competition is expressed by the spite (ρ_s) and competition (ρ_c) density, respectively. Together with the spite genotype ϕ_i, these are used to calculate growth and death probabilities for each individual, as detailed below.

2.2 Model variants

2.2.1 Stepwise model. We studied a stepwise model which allowed spite genotypes ϕ_i to vary in small intervals with constitutive spite expression and release. The initial spite levels were set to zero and fluctuated over time until they reached an evolutionary equilibrium. To prevent mutational bias toward positive values, spite genotypes were also allowed to become negative. For any other calculations, these were treated as zero, with no effect on growth and death rates. In the first set of simulations, each individual was immune to toxin produced by itself (no self-spite), but susceptible to spite from others. Although this scenario is unrealistic when modeling diffusible bacteriocins, it is plausible for other types of spiteful interactions, such as delivery through long range membrane protrusions. Experiments were also run where individuals were susceptible to their own spite production (self-spite), and the final set of simulations introduced increased toxin resistance to spiteful individuals in addition to self-spite.

2.2.2 Dichotomous model. A dichotomous model was implemented where individuals were either carriers or non-carriers of a spite plasmid with $\phi = 0.5$ and initial frequency $f_0 = 50\%$ for most simulations. Carriers were assumed to be much more resistant than non-carriers. Spite release was lethal requiring individuals to burst, which occurs with a given spite expression probability. This mechanism is biologically inspired by colicins and pyocins from gram-negative bacteria [34, 40].

2.3 Growth

Individuals reproduce asexually, generating a newborn with the same spatial coordinates. The chance to divide at each time step is calculated as $\psi_i = \psi_0(1 - \rho_{c,i}/k_c)(1 - C\phi_i)$. This is modelled by logistic growth, with a basal growth rate $\psi_0 = 1$ that is decreased by the competition density ρ_c relative to the maximal carrying capacity k_c. ρ_c represents the local competition density for resources and reduces the growth rate to zero when it is equal to or exceeds the carrying capacity. Spiteful individuals also pay a percentage of their growth rate as a metabolic cost determined by multiplying the cost coefficient C with their spite genotype ϕ_i. Note the effective fitness cost of spite depends not only on the metabolic costs, but also on its effects on individual death rates.

2.4 Mutation

Newborn individuals can undergo mutations changing ϕ_i. In the stepwise model, this occurs with a mutation probability of 1% and a mutation step of 0.05. In the dichotomous model, newborn individuals have a 1% chance to obtain or lose the plasmid with a corresponding spite level of $\phi = 0.5$.

2.5 Death

The probability of dying in a time step was calculated as $\delta_i = \delta_0(1 + \rho_{s,i}/k_{d,i})$. Individuals have a basal death rate δ_0 of 0.1 and this is increased by the spite density ρ_s relative to the spite tolerance k_d. Runs with resistance were implemented by adding ϕ_i to the individual tolerance $k_{d,i} = k_d + \phi_i$.

2.6 Dispersion

Individuals undergo diffusion at each time step with a diffusion constant D, being displaced in the x and y coordinates by normally distributed distances with variance $\sigma_d^2 = 2D\Delta t$ and standard deviation σ_d. Importantly, dispersal is divided in two steps after the growth and death phases, each with variance $\sigma_d^2/2$ and standard deviation $\sigma_d/\sqrt{2}$. To discretize this diffusion process, displacements are chosen from integers within the range of [-6, 6] standard deviations, where the probability for each integer Z_i is equal to the density sum of the normal distribution in the interval $[Z_i - 1/2, Z_i + 1/2]$. To simulate well-mixed models where $\sigma_d \to \infty$, individuals are placed in randomly drawn positions during each diffusion step.

2.7 Kernel density estimates

Spite and competition densities ρ_s and ρ_c are determined using Gaussian kernels, denoted as $K(\Delta S|\sigma)$. The contribution of each individual j towards the density ρ of an individual at position S is calculated by taking their Euclidean distance (ΔS) and applying the Kernel function $K(\Delta S|\sigma)$. For $\rho_{s,i}$, ϕ_j also weighs the kernel contribution to the density. Therefore, the total competition and spite density an organism experiences are:

$$\rho_{c,i} = \sum_{j \neq i}^{N} K\left(\|S_i - S_j\| \mid \sigma_c\right)$$

$$\rho_{s,i} = \sum_{j \neq i}^{N} K\left(\|S_i - S_j\| \mid \sigma_s\right) \phi_j$$

We assume individuals do not compete with themselves, excluding $j = i$ from the summation. Self-spite $i = j$ was included after the first set of simulations. Note σ_c and σ_s are the standard deviation of the Gaussian kernels, and therefore control the strength of interaction between individuals at different distances. A larger σ means an individual can impact a larger area, but his influence on the local density is also diluted.

As the field has periodic boundaries, the densities are also influenced by the implicit parallel projections of the field. This means each position S_i is affected not only by individuals at positions S_j but also mirror images after wrapping around the edges at $S_j + n(L, 0) + m(0, L)$, $n, m \in \mathbb{Z}$. Although there are infinite projections, the contributions in the Gaussian kernel become negligible as they get further away, so this was approximated by considering 3 projections on all directions.

Table 1: Fixed Parameter Settings. L: Field size, n_0: initial number of individuals, p_{mut}: probability of mutation, ψ_0: basal growth rate, δ_0: basal death rate.

L	n_0	p_{mut}	ψ_0	δ_0
512	13,000	0.01	1	0.1

To calculate the density at each position in the lattice, accounting for each individual contribution and the parallel field projections, we made use of Discrete Fourier Transforms and the Circular Convolution Theorem. To achieve this, the Fast Fourier Transform (FFT) of the kernel function matrices is calculated once before the simulation loop. At each time step, a matrix with the individual contributions for the spite and competition density is obtained. A FFT is applied and this is multiplied by the FFT of the kernel. Applying the inverse FFT to this product returns the convolution with the desired field density at each position.

2.8 Parameter Combinations

To study the effects of the spatial scales and other parameters, we used a semi-factorial design. The diffusion constant was chosen to obtain a σ_d of 4, 8, 16 and $i = \infty$. σ_s and σ_c were determined relative to σ_d , varying it by 2 orders of magnitude in the $\log_2$ scale. For $\sigma_d \to \infty$, factorial combinations of 2, 4, 8, 16 and 32 were used. This parameter sweep was performed with multiple other parameter combinations as detailed in tables 1 and 2.

2.9 Computer simulations

To perform computer simulations, space and time are treated discretely with units Δx and Δt. These can be rescaled to represent any unit of physical space and time, which requires rescaling all other parameters appropriately. For simplicity, we always treated these as 1, allowing us to omit them from model expressions above. Simulations are initialized with $n_0 = 13,000$ individuals in the field, near the maximum allowed by density regulation with a carrying capacity $k_c = 0.05$, $L^2 k_c \approx 13,000$.

For the stepwise model, simulations were run for $t_{max} = 250,000$ time steps and the average spite $\bar{\phi}$ was measured over the last 150,000 steps. For the dichotomous model, the spite level was fixed and only the carrier frequency needed to equilibrate. This allowed us to use much shorter simulations $t_{max} = 1,000$ or $10,000$, averaging results over the last 100 time steps.

3 RESULTS

3.1 Stepwise model

The first set of simulations was performed with the stepwise model, with constitutive toxin expression and stepwise mutations. Individuals were immune to toxin themselves produced, but not resistant to spite produced by others. Under these conditions, only a small region of parameter space evolved allelopathy, measured as having a positive average spite genotype ϕ in the last 150,000 steps (Fig. 1A).

It has been shown that spatial structure can favor the evolution of toxin production [6, 21]. In spatially homogeneous or well-mixed systems, competition is global, so any benefits generated by decreased competition are spread evenly across individuals. Since spiteful individuals also pay a constitutive metabolic growth rate cost, it becomes challenging for it to be positively selected. This would require having enough toxin producers in the population to eliminate most susceptible individuals, with either a sufficiently high killing efficiency or producer frequency [17]. Consistent with theory, we did not observe positive selection for spite with $\sigma_d \to \infty$ (Fig. 1B). Additionally, If σ_d is high enough, the model converges to a well-mixed setting, where most spatial relationships become weak. Our results show that $\sigma_d = 16$ was enough to disfavor evolution of allelopathy in our model.

The number of individuals in the field depends on the effective carrying capacity. Although this is partly set by the k_c parameter, the effective carrying capacity also depends on the death rates and therefore the toxin levels that evolve. The number of individuals generally stayed close to the maximum allowed by the growth carrying capacity $k_c = 0.05$, which was expected to be $L^2 k_c \approx 13,000$. Notably, decreasing σ_c below 4 caused a density regulation break, as the standard deviation of the Gaussian kernel became too small introducing granularity artifacts (Fig. 1C). This caused a confounding increase in field density along with decreasing σ_c , which occurs in the last rows of the $\sigma_d = 4$ and $\sigma_d = 8$ panels. Although this was unintended, it had the benefit of probing density-related effects within the same parameter sweeps. Positive spite values were expected to evolve with lower values of σ_c , as local competition should help promote spite evolution [6, 14, 28]. This was not observed at $\sigma_c = 1$, which is likely an effect of increasing the field density (Fig. 1A).

Importantly, in these first simulations no kin discrimination was implemented. This means toxin producers were not resistant and the toxin was not preferentially targeted at non-producers. In these conditions, excessively viscous environments could impair allelopathy selection, as there would be a greater spatial assortment of genotypes and spiteful individuals would interact more frequently with their own kin (higher relatedness) [37, 42]. To investigate this, we extended the range of σ_d using combinations of σ_c and σ_s equal to 4 or 8, which previously yielded the highest $\bar{\phi}$ (Fig. 1A). Indeed, further decreasing σ_d led to smaller or negative average toxin genotypes (Fig. 2A).

An important caveat was that individual toxin contributions towards their own density were initially excluded. This led to the suspicion that individual producers were surviving and benefiting from decreased competition created by their toxin. While this behavior is often found in nature, the individual fitness costs would be negative on average and it should be classified as selfishness, not spite. Indeed, when self-spite was included in the ρ_s density summation, this prevented the evolution of toxin production (Fig. 2B). In this setting, the toxin contribution of an individual is highest at its own position, contributing more towards killing itself than neighboring individuals. Clearly, the competitive benefits to related individuals did not outweigh the individual costs.

Consistently, when excluding self spite we did not detect negative relatedness in simulations where allelopathy evolved (Fig 1A,

Table 2: Variable Parameter Settings. k_c : carrying capacity, C: cost coefficient, k_d : baseline toxin resistance, s_{mut}: mutation step, t_{max}: number of iterations, f_0: initial carrier frequency, Spite Exp.: spite expression probability.

Model	k_c	C	k_d	s_{mut}	t_{max}	f_0	Spite Exp.
Stepwise	0.05	0.005	0.05, 0.05 + ϕ_i	0.05	250,000	0	1
Dichotomous	0.05, 0.25	0.01, 0.1	[0.001, 0.002, 0.005, 0.01]+ ϕ_i	0.5	10,000	0.5, 0.25, 0.1, 0.02	0.001, 0.01, 0.05, 0.2

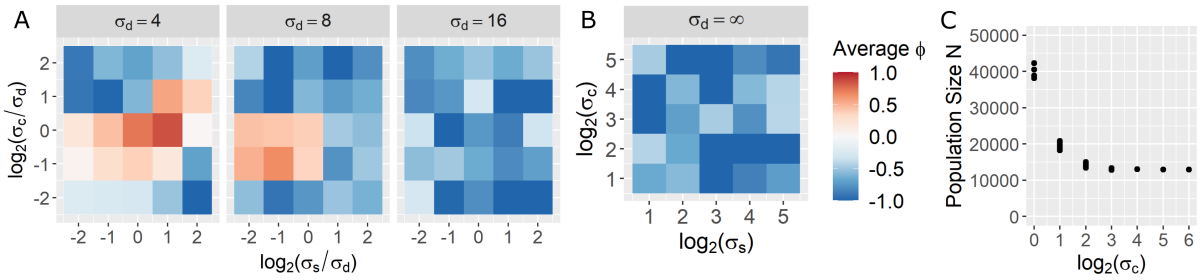

Figure 1: Stepwise model only evolved allelopathy in viscous environments. Final average ϕ obtained when (A) varying σ_d and (B) in the well-mixed model $i = \infty$. The competition σ_c and spite σ_s ranges were varied relative to diffusion range σ_d in base 2. The x and y axis are expressed as the log_2 of the ratio between σ_c or σ_s and σ_d , respectively. The corresponding σ_d is indicated on the top of each panel. (C) Number of individuals in the field as a function of σ_c .

Figure 2: Effects of diffusion, self-spite and resistance on the stepwise model. Final average ϕ obtained when (A) extending the diffusion σ_d range (B) including individual sensitivity to their own spite and (C) including resistance to spite proportional to their toxin production. Colors represent combinations of spite σ_s and competition σ_c ranges. Shapes represent the cost coefficient per unit of toxin production.

2A). Relatedness was measured as the $\text{Cov}(\rho_s, \phi_i)/\text{Var}(\phi_i)$, which can be derived using a variant of Hamilton's rule from a linear regression between fitness w and the spite genotype ϕ_i [32]. This means higher spite levels were generally associated with a higher spite density, so relatedness was mostly positive between interacting individuals, working against the conditions for spite selection $RB > C$. To obtain a negative relatedness, spiteful individuals would need to interact more often with individuals with lower spite levels, which would likely require particular spatial and dispersion patterns, such as chemotaxis. In theory, this could also be achieved by having small competition ranges, so that interactions are local between few individuals. When individuals exclude themselves from the density summation, they also interact with one less individual, although this was not enough to yield negative relatedness in our simulations.

Another mechanism for increasing negative relatedness is adding kin discrimination [12]. By selecting interaction partners (negative assortment) or increasing the resistance of toxin producers, they interact less intensely, and with full immunity they do not interact at all. Therefore, interactions towards non-producers become relatively stronger, favoring allelopathy selection. We included toxin resistance by adding the individual ϕ_i to k_{d_i}. Much higher spite values evolved with kin discrimination (Fig. 2C). This was mostly limited by the metabolic cost coefficient C, and positive values still

evolved even with a 20-fold higher cost $C = 0.1$.

Interestingly, this seems to be an asymmetry with altruistic behaviors, where kin discrimination is not a requirement [2]. Although it becomes susceptible to cheaters, public-good production can still evolve without different interaction strength between individuals based on their phenotype. However, it is still required to have some degree of positive assortment, with more frequent interactions between cooperators, or having semi-isolated groups with different producer frequency in a meta-population [7].

3.2 Dichotomous model

Even though self-spite was maintained for simulations with resistance in the stepwise model (Fig. 2C), the selfishness caveat remained as producers also had increased toxin resistance. Therefore, they were still likely to survive and benefit from decreased competition. To address this, we developed a dichotomous model that includes lethal spite expression, similar to colicins [20, 38, 40]. The model was dichotomized to contain only carriers with $\phi = 0.5$ and non-carriers. Producers still had constitutive metabolic costs, but were required to burst to release toxin in the medium. This guaranteed spiteful individuals did not get direct fitness benefits and paid the ultimate fitness cost of self-sacrifice in addition to having lower growth rates. In this scenario, constitutive spite expression would not be viable as it would instantaneously eliminate all toxin producers. Therefore, a spite expression probability parameter was introduced, controlling the probability of bursting at each time step. This was set to 0.1%, 1%, 5% and 20%, a range consistent with experimental measurements [17, 41].

As only a fraction of individuals would burst in each time step, spite densities would generally be lower than what is obtained with constitutive expression. To ensure that individual bursts would still be effective against non-carriers, the k_d was lowered to 0.001. The cost coefficient was set to 0.01 or 0.1, which corresponds to producer relative growth rates of 99.5% and 95%, respectively. Results are shown only for $C = 0.1$, with 95% of the growth rate of non-producers. As only the carrier frequency was required to equilibrate, this allowed a larger number of simulations for a shorter time ($t_{max} = 1,000$ or $10,000$). The initial frequency f_0 was initialized at 0.5 for most simulations. Although this does not model the initial evolution of a spite-encoding gene as it assumes a high initial carrier population, understanding in what conditions toxin producers can thrive is helpful in providing evolutionary insight.

Interestingly, larger spite expression probabilities facilitated the selection of carriers, despite the higher fitness cost of the increased death rate, even at 20% (Fig. 3). When expression was 0.1%, carriers were not selected as the rate at which they eliminated non-producers was too low to compensate for their metabolic costs. By increasing the burst rate, they could rapidly eliminate non-carriers in most settings, but this depended critically on the spite range. Carriers were usually disfavored when σ_s was small and lower than the competition range σ_c . This can be noticed by observing the secondary diagonal in each subpanel, which corresponds to $\sigma_s = \sigma_c$. This result makes sense as having a competition range much larger than the toxin range should importantly decrease the efficiency of toxin release in reducing local competition for other carriers. Results for a cost of 0.01 were generally similar, but producers won in a larger area of parameter space.

Notably, there were still regions where producers could win in the well-mixed model, with infinite diffusion (Fig. 3B). Even though spatial structure is important to facilitate selection of toxin production, they can still thrive if the frequency of producers and killing efficiency is large enough [6, 17, 21].

The regions with the smallest σ_c were also beneficial for toxin production, which are found at the last rows of the $\sigma_d = 4$ and $\sigma_d = 8$ panels. Importantly, the model still had the same confounding density effect (Fig. 1C), which was indicative of field density as a contributor for producer selection. Indeed, the total number of bursting producers is what determines spite densities on the field, and this depends on the total number of individuals, carrier frequency and spite expression probability. This is consistent with experimental work, where density and frequency dependency was found for relative growth advantages of yeast and bacteria toxin production [6, 21].

Altogether, these results indicate there is a nucleation barrier for producers to win. They need to overcome a frequency threshold to become fixated in the population, and this value is affected by the various model parameters. If this value is of order 1, then carriers or non-carriers can invade an homogeneous population from rare. Naturally, a single isolated producer can only invade if it generates a small colony before bursting, otherwise there would be no related individuals to benefit from the decreased competition. To further investigate how various model parameters affected the selection for toxin production, we varied the starting frequency f_0, the population baseline resistance k_d and carrying capacity k_c .

Decreasing the initial carrier frequency f_0 to 0.25, 0.1 and 0.02 greatly reduced the area where producers could win (Fig. 4). This was mostly restricted to the 1% and 5% spite expression panels, and in the last rows of $\sigma_d = 4$ subpanels. Results for $f_0 = 0.1$ are similar to $f_0 = 0.25$ and not shown. Carriers could no longer fixate In the well-mixed model, which is consistent with the difficulty to select spite without spatial structure. Producers have a lower growth rate and increased death rates from spite expression, but the decreased competition they create is shared equally among surviving individuals. In spatially homogeneous environments, the fitness benefits to carriers rarely outweigh these costs, especially with lower producer frequencies. There was a particular region where carriers could still invade from rare, but this was mostly restricted to a lower metabolic cost of 0.01 (99.5% growth rate), 1% spite expression and a higher field density ($\sigma_c = 2$) (not shown).

The selection of producers was also extremely sensitive to the population baseline resistance (Fig. 5). Increasing the k_d from 0.001 to 0.002 dramatically shrunk the area where producers won only to the last row of the $\sigma_d = 4$ panels, where there is the smallest σ_c and a higher field density. Producers never thrived when a higher k_d of 0.005 and 0.01 was used. Toxin effectiveness is therefore crucial for

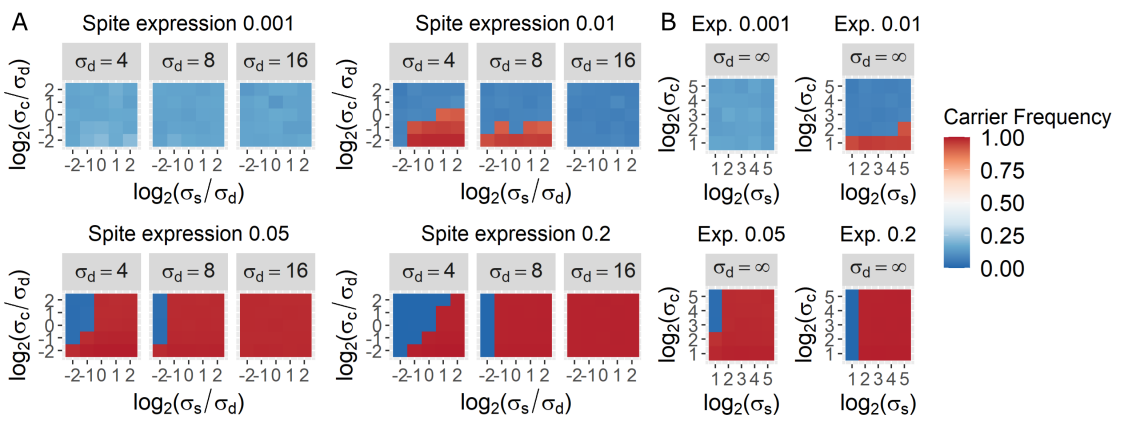

Figure 3: Spite is selected with higher expression probabilities and spite range in the dichotomous model. Final carrier frequency obtained in the dichotomous model when (A) varying σ_d and (B) in the well-mixed model $i = \infty$. The competition σ_c and spite σ_s ranges were varied relative to diffusion range σ_d in base 2. The x and y axis are expressed as the log_2 of the ratio between σ_c or σ_s and σ_d , respectively. The corresponding σ_d is indicated on the top of each panel. In the $\sigma_d \rightarrow i$ panels, spite expression is abbreviated to Exp.

the selection of spite in this model.

Jointly, the $\sigma_c = 2$ results with a population size $N \approx 40,000$ strongly indicated there were important field density effects. To investigate this, we performed simulations with a higher carrying capacity $k_c = 0.25$, which allowed between 50,000 and 100,000 individuals in the field. Increasing the total field density allowed producers to win in almost all parameter combinations, even with the lowest spite expression levels (Fig. 6A). As the main determinant of toxin concentrations is the number of bursting individuals, spite densities were larger overall, efficiently eliminating most non-carriers.

We then asked if increasing the baseline tolerance would revert this effect. When using $k_d = 0.005$, carriers won over most combinations or lost completely depending mostly on the spite expression probability (Fig. 6B). Importantly, spite was never selected before with $k_c = 0.05$, so higher field densities also allow for considerably higher population toxin resistance. Additionally, it is required to have a proper balance between the death rates and effective toxin concentrations, as carriers only won with 1% and 5% spite expressions.

Our final set of simulations sought to answer whether the density increase could help producers to invade from rare, using an initial frequency of 2% (Fig. 7). Even with a low starting frequency, producers were positively selected over many parameter combinations, as opposed to lower densities (Fig. 4). They dominated regions with spite expressions of 1 and 5%, except in the lowest spite ranges σ_s . Interestingly, carriers were usually not selected with the highest expression rate of 20%, which increases the death rate of producers excessively without eliminating enough non-carriers due to the low frequency. For the lowest spite expression, producers could still thrive, but mostly in regions with higher viscosity (lower σ_d). Increasing the dispersion rates reduces the strength of spatial relationships, which are likely more important when the producer frequency is low.

4 DISCUSSION

Our model provides an intuitive framework for studying spatial scales of interaction by explicitly modeling the ranges of resource competition, spiteful interactions and individual dispersal. By using a minimal model of allelopathic spite selection, the impact of these parameter manipulations become readily interpretable. Our results are highly consistent with previous model predictions and experimental work in bacteriocin toxin production, as we will discuss in the sections below. We briefly review a selection of existing models and experiments of allelopathic interactions, outlining their approach and main results. It is important to note that some allelopathy models are not concerned with discerning spite from selfishness, so producers are generally immune and can still obtain direct competitive benefits. Nevertheless, they are still informative for understanding selection of allelopathic spiteful behaviors.

4.1 The effect of the scale of competition and producer frequency on the strength of selection

It has been predicted that spiteful strains would be optimally selected when at intermediate frequencies in the population. Also, the scale of competition should play an important role, determining how often spiteful individuals compete with the local neighborhood affected by spite production or with individuals from the global meta-population.

Gardner, West and Buckling [15] modeled mixed spiteful community patches with different bacteriocin-producing strains, where

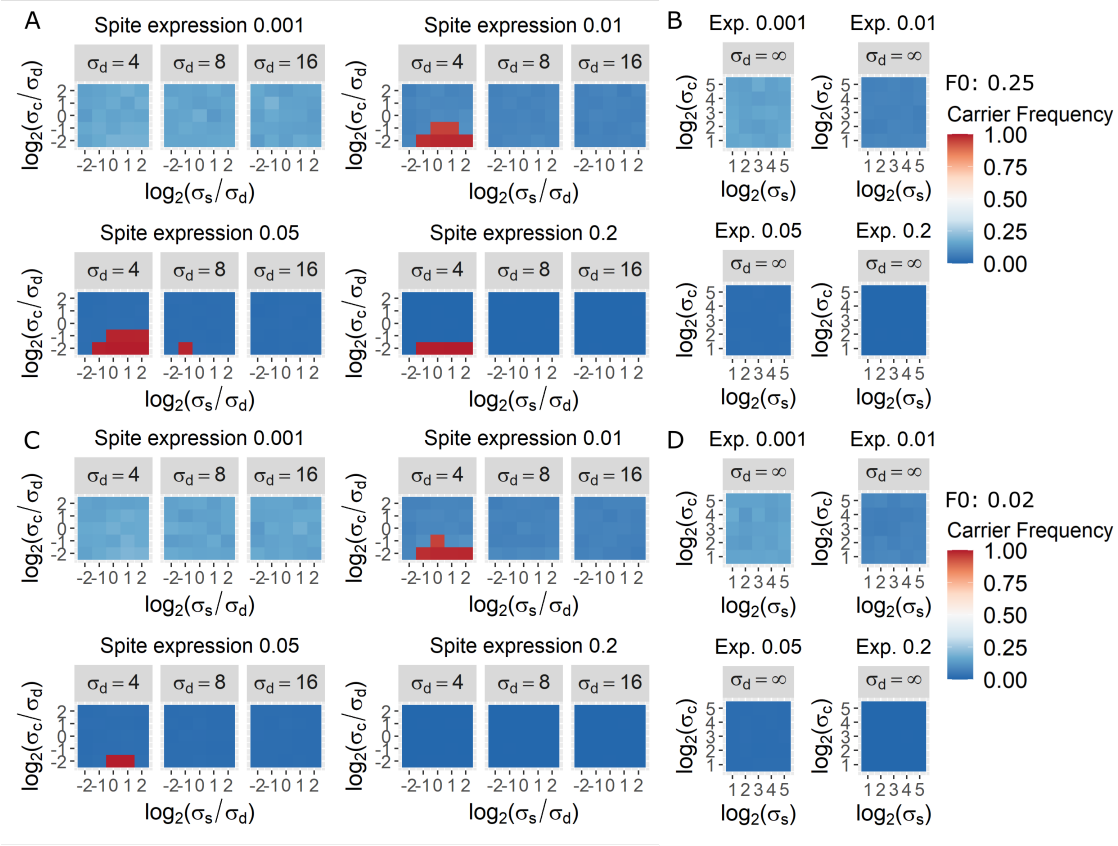

Figure 4: Selection of producers is highly dependent on initial frequency. Final carrier frequency obtained in the dichotomous model for starting frequencies (A,B) 0.25 and (C,D) 0.02. The competition σ_c and spite σ_s ranges were varied relative to diffusion range σ_d in base 2. The x and y axis are expressed as the log_2 of the ratio between σ_c or σ_s and σ_d , respectively. The corresponding σ_d is indicated on the top of each panel. In the $\sigma_d \rightarrow i$ panels, spite expression is abbreviated to Exp.

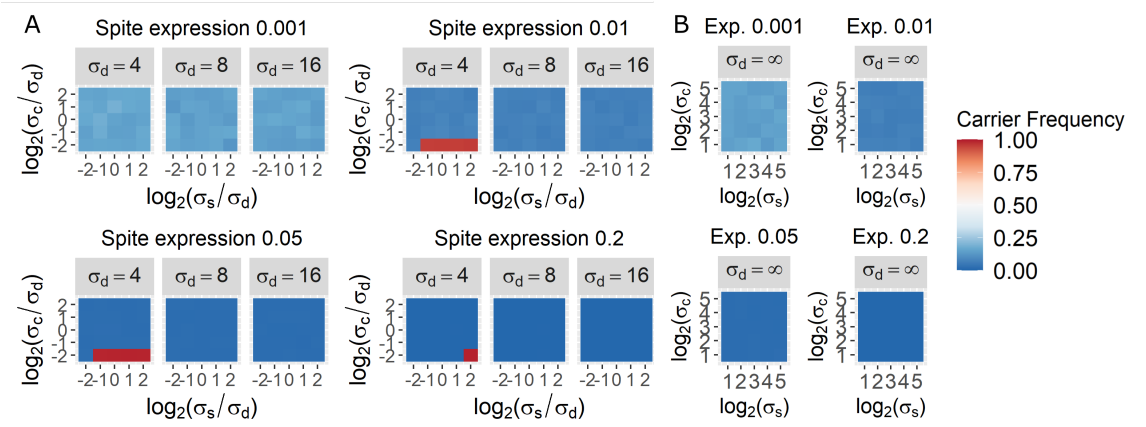

Figure 5: Increasing toxin resistance eliminates advantage of producers. Final carrier frequency obtained in the dichotomous model when (A) varying σ_d and (B) in the well-mixed model $i = \infty$. The competition σ_c and spite σ_s ranges were varied relative to diffusion range σ_d in base 2. The x and y axis are expressed as the log_2 of the ratio between σ_c or σ_s and σ_d , respectively. The corresponding σ_d is indicated on the top of each panel. In the $\sigma_d \rightarrow i$ panels, spite expression is abbreviated to Exp.

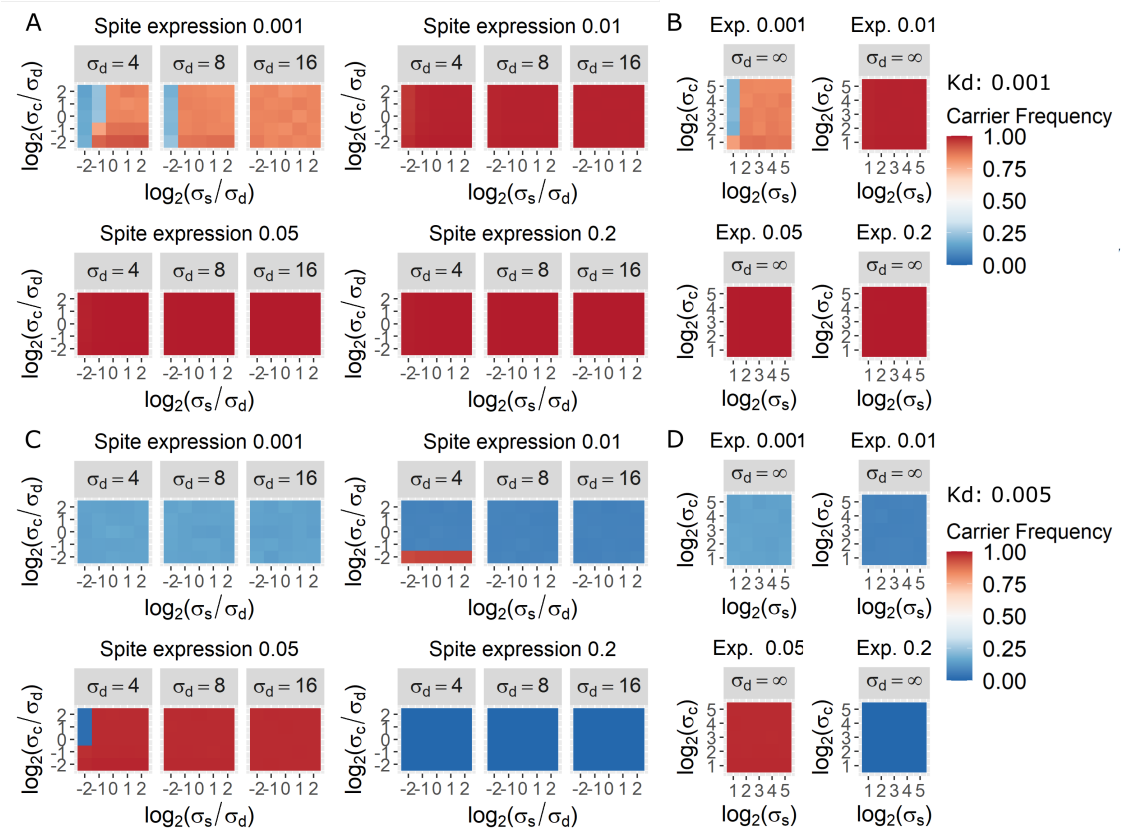

Figure 6: Increasing the field density strongly benefits toxin-producers and allows selection even with higher toxin resistance. Final carrier frequency obtained in the dichotomous model with $k_c = 0.25$ for k_d (A,B) 0.001 and (C,D) 0.005. The competition σ_c and spite σ_s ranges were varied relative to diffusion range σ_d in base 2. The x and y axis are expressed as the log_2 of the ratio between σ_c or σ_s and σ_d, respectively. The corresponding σ_d is indicated on the top of each panel. In the $\sigma_d \rightarrow i$ panels, spite expression is abbreviated to Exp.

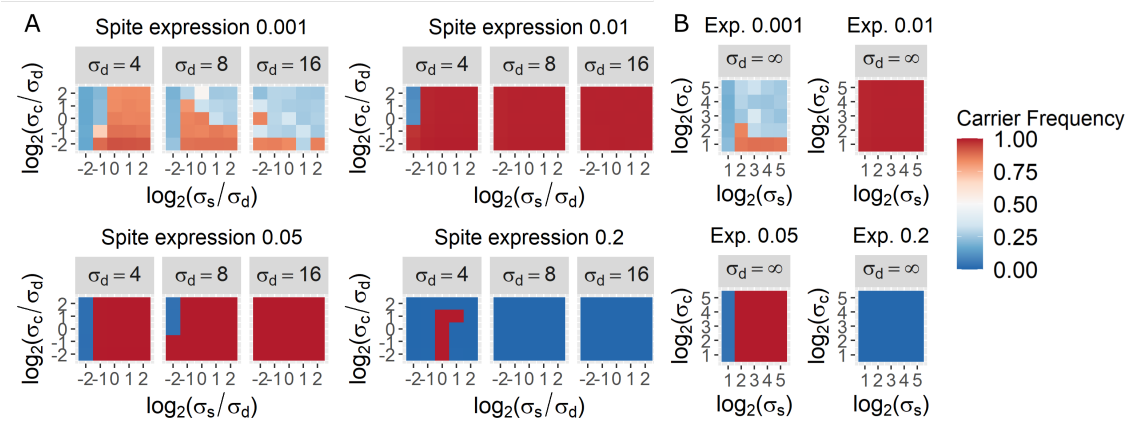

Figure 7: Increasing the field density allows toxin-producers to invade from rare. Final carrier frequency obtained in the dichotomous model when (A) varying σ_d and (B) in the well-mixed model $i = \infty$ with $k_c = 0.25$ for $f_0 = 0.02$. The competition σ_c and spite σ_s ranges were varied relative to diffusion range σ_d in base 2. The x and y axis are expressed as the log_2 of the ratio between σ_c or σ_s and σ_d, respectively. The corresponding σ_d is indicated on the top of each panel. In the $\sigma_d \rightarrow i$ panels, spite expression is abbreviated to Exp.

lineages are immune to toxins they produce but susceptible to others. Each strain experiences a growth rate reduction given by the cost of resources allocated to toxin production and spite from other lineages. Taking one strain as a reference, its proportion in each community was determined by the kinship parameter r. A scale of competition parameter a determined the proportion of interactions which are local versus taken randomly from the population. The authors then measure the investment in bacteriocin production that maximizes the fitness of the reference strain. The fitness is defined as the ratio between the focal lineage growth rate and the weighted average of local and global population growth rates.

The model predicts the highest spite levels should evolve with kinship $r \approx 0.5$ and intense local competition (higher a). This result can be understood intuitively: When the degree of kinship tends to zero, toxin production is less effective in reducing the local competition of other strains and relatedness is only weakly negative. When kinship tends to one there exists only a small density of susceptible cells to affect, so toxin production becomes less cost-efficient in reducing competition within the patch. If competition is mostly local, spite production has more relative impact on the fitness of competing strains. With lower values of a, there is more global competition taken from the average population, decreasing the importance of local bacteriocin production. In summary, their results show the optimal investment in toxin production is maximized when competition is local and the focal strain frequency is intermediate within communities. A very similar model was studied by Inglis et al. (2009), drawing the same conclusions [27].

The models by Inglis et al. (2009) [27] and Gardner et al. (2004) [15] assumed that spiteful strains are vanishingly rare in the global population, but this could have interactions with the effect of the scale of competition. This limitation was addressed by Inglis et al. (2011), which extended a similar model to handle higher global spite frequencies in the meta-population [28]. Individual patches were modelled with either one (clonal) or two founding individuals. Spite is never selected if patches are clonal, as producers always have lower intrinsic growth rates. If patches are founded by two individuals, the fitness depends on the global spite frequency of the population p, the scale of competition a, the spite toxicity k and cost c. Spite can only be favored if the growth rate decrease in susceptible cells is larger than the growth rate production cost, that is, if $k > c$. Fitness of producing strains increases monotonically with a if $p < (c + k)/2k$, is independent of a at $p = (c + k)/2k$ and decreases with a if $p > (c + k)/2k$. This point is $p \approx 0.5$ when $k \gg c$. Therefore, the effect of the scale of competition depends on the meta-population spite frequencies p. Fitness decreases monotonically with p if competition is purely local $a = 1$ and is a dome shaped quadratic function of p otherwise. These results show that local competition should favor spite the most if producer frequencies are below $\approx$ 50%, assuming the toxin is efficient.

Additionally, the authors performed a meta-population experiment with bacteriocin producing (PAO1) and non-producing (O:9) *P. aeruginosa* strains, where they were grown manipulating spatial relatedness and the scale of competition [28]. These strains underwent multiple serial cultures, alternating between well-mixed tubes for 18h and agar plates for 18h. High spatial relatedness was obtained by inoculating starting tubes with single isolated strains (50% of the tubes each), and intermediate relatedness by mixing strains 1:1 in the first generation. Global competition was set by mixing colonies in a single tube in between platings, while local competition allowed randomly sampled colonies to grow alone in tubes. Bacteriocin producers increased in frequency only when in intermediate relatedness and were favored by local competition, consistent with theoretical predictions.

The same design was used by Griffin et al. (2004) to study cooperation in *P. aeruginosa*, where costly production of siderophores benefits the whole population for iron-scavenging [22]. This meta-population experiment was conducted between PAO1 strains and PA06609, a mutant derived from PAO1 that does not produce the siderophore pyoverdin. Interestingly, their results show that global competition and high relatedness benefit selection of cooperators, the opposite of spiteful bacteriocin selection.

In our models, the scale of competition is controlled by an interplay of the spatial scales of interaction. The degree of local competition is increased by having limited dispersal and small competition ranges. Consistent with theoretical predictions [15, 27, 28], these were the regions that generally promoted spite selection.

In our dichotomous model we started most simulations at 50% carrier frequency. This intermediate kinship should increase the relative benefit of spite expression. However, as spiteful traits undergo positive or negative selection, their frequency readily changes, moving away from the theoretical optimum at $\approx$ 50%. Therefore, in our dynamical models this is a condition that cannot be maintained over time and purifying selection tends to drive the carrier frequencies to extreme values. Growth rate advantages to producers at different frequencies would only be measurable in brief simulations, before the field converges to steady state frequencies at the extremes.

4.2 Spiteful interactions and their impact on virulence

Social interactions within parasitic infections can importantly affect the course of disease [5]. Therefore, the prediction that intermediate relatedness should favor spite has implications for virulence of parasite infections that contain allelopathic interference competition. This also has important consequences for bacteriocin interactions in industrial settings and food spoilage [31].

In the aforementioned work, Gardner, West and Buckling also modeled parasitic virulence by assuming host mortality increases with the average population growth rate [15]. Virulence represents the reduction in host survival and is highest at the extremes of kinship $r = 0$ or 1. In these settings, interference competition is minimized, increasing the growth rate of strains in the infection. At intermediate frequencies, higher levels of spite are promoted which lowers the overall population growth rate and host mortality.

These predictions were validated experimentally with *P. aeruginosa* competition assays and infections in caterpillars [27]. Consistent with their theoretical model [27], the selection coefficient for the bacteriocin-producing strain PAO1 in competition with the non producer O:9 is highest when grown in intermediate starting frequencies. Virulence was minimized in mixed infections with intermediate frequencies (50%) compared to 1% and 99%, as measured in post-infection time of death of caterpillar hosts.

Similar work was conducted by Massey et al. (2004) competing *Photorhabdus* Asy and Hb and *Xenorhabdus* X3 bacteria, nematode-symbionts that are parasitic in caterpillars and produce bacteriocins [33]. In mixed infections of caterpillar hosts, both Asy and X3 competitively excluded Hb with growth inhibition. Consistently, combined infections of Hb with Asy or X3 had similar virulence as single infections, as Hb is competitively eliminated. Mixed infections of Asy and X3 did not lead to competitive exclusion and had lower virulence, which was attributed to spiteful interference competition between them.

Vigneux et al. (2008) performed studies of virulence by infecting caterpillars with nematodes *Steinernema carpocapsae* and their symbiont bacteria *Xenorhabdus nematophila* under different nematode migration treatments [43]. High spatial relatedness was maintained through low migration by experimentally infecting hosts with 90% of worms emerging from the same host. Low spatial relatedness (high migration) was obtained by mixing the infective dose from 8 different hosts. The low migration treatment led to more virulent infections, measured by the percentage of live hosts over time and this was associated with mild interference competition. Compared to the low migration regime, the high migration treatment was less virulent and it was more likely to observe growth inhibition between bacterial isolates from one or multiple hosts.

A consistent result was obtained by Hawlena et al. (2010), who performed an experiment extracting entomopathogenic nematodes from soil samples at different distances [26]. They demonstrated that larger physical distances of bacteriocin producing communities was associated with lower genetic relatedness and higher probability of growth inhibition.

In summary, the selection for spiteful interactions can impact the course of disease in parasitic infections. The frequency of toxin producing strains is important to determine the relatedness between individuals and the relative competitive advantage of expressing spiteful behaviors. By decreasing the overall population growth rate, allelopathic toxin production tends to reduce virulence.

4.3 Frequency and Density Dependence for spite selection

The ability for strains to invade populations from rare is considered an important attribute to maintain ecological diversity. However, it can be challenging for toxin producers to fixate from low initial frequencies, as toxin concentrations tend to be small. Toxin concentrations can be increased by having higher cell densities or viscous spatially structured environments, so that diffusivity is low and interactions are mostly local.

Steven Frank [13] worked on allelopathy models of well-mixed environments. He formalized spatially homogeneous models with ordinary differential equations (ODEs) describing immune producers, susceptibles and toxin abundances through a dimensionless analysis. These well-mixed models always flow to pure producers or pure susceptibles and when equilibrium at intermediate frequencies exist, it is unstable and corresponds to a saddle point. Notably, the species with higher initial frequency always have a significant competitive advantage, but it is still possible for the less abundant species to thrive depending on model parameters. The separatrix determining the winner is dislocated by the dimensionless toxin cost, decay rate and toxicity relative to resource-dependent competition, which is proportional to the carrying capacity and therefore the field density.

Durret and Levin 1997 also worked on an ODE model with similar results [10]. Their well-mixed system always flows to pure producers or susceptibles with important advantages to the most common species. They proceeded to introduce a spatial model with grid points that could be occupied by susceptibles or producers, and the transitions between grid states was determined by the neighborhood occupancy, growth and death rates. In contrast with well-mixed systems, producers became able to invade from rare (1%) with spatial structure, also depending on the relative growth rates, death rates and toxicity.

Chao and Levin 1981 showed experimentally that ColE3 colicinogenic bacteria could only invade sensitive populations in well-mixed environments from relatively high initial frequency above 2%. If however, there is spatial structuring in a viscous agar environment, toxin production is more advantageous locally, and producers invaded from frequencies as low as 10^{-6} [6].

Greig and Travisano 2008 performed competition experiments between *S. cerevisiae* susceptible and toxin-producing yeast strains, varying the culture density in addition to the frequency of producers [21]. These toxins are encoded by dsDNA K1 viruses and are secreted instead of requiring cells to burst. The relative fitness of producers was strongly dependent on their initial frequency and density, both in liquid (well-mixed) and solid (viscous) cultures. Unlike the bacteriocin results [6], yeast toxin producers could not invade from rare, only had advantages at high starting frequencies (50%) and this required densities above 10^{-3} of the stationary phase. Advantages of producers were more pronounced in solid cultures than well-mixed but still required high frequencies and densities.

Adam et al. (1979) performed competition experiments in mixed environments between parental and ColE1 *E. coli* and report a similar initial frequency required for selection of producers ($\approx$ 50%) [1]. The authors discuss that this critical value should differ between colicin types and environments, and that it should be lowered by increasing the cell density.

Altogether, these experiments and predictions are highly consistent with our results. Producers generally won when starting

at 50% frequency, but this depends on other model parameters to determine the cost-efficiency of toxin expression. When starting from low starting frequencies (2%), producers usually could not invade, especially if spatial relationships are destroyed in the well-mixed model. If the field density is increased however, the ability of producers to dominate is generally rescued.

4.4 Spite expression and regulation

In our dichotomous model, spite expression was stochastic and controlled by bet-hedging a probability to burst. This parameter can be interpreted as the penetrance of the spiteful phenotype and it played an important role in determining the advantage of producers. When it is too low, the efficiency of clearing competitors decreases and may not compensate for the intrinsic metabolic costs. When it is high, spite densities increase benefiting carriers, but it also represents an importantly increased death rate.

Experimental work by Ghazaryan et al. (2019) introduced colicin A, E2, E7 and E8 plasmids with different expression levels in otherwise non-producing *E. coli* strains [17]. Competition experiments were performed varying the initial frequency of producers in well-mixed environments. The producer strains had lower growth rates than the parental non-producing strains, but when cultured in competition with another susceptible strain they enjoyed a fitness advantage over non-producers. The authors show that higher colicin expression rates are associated with a lower initial frequency required to compete with sensitive cells and with a lower non-producer survival, which is consistent with our spite expression results (Fig. 3). An important limitation was that the comparison of different expression rates was made between different colicins, which have other distinct properties. Controlling the expression of a single colicin type with inducible systems would allow to better isolate the effect of spite expression.

Importantly, many bacterial communities are able to regulate the proportion of carriers that express bacteriocins [8, 18, 20]. Many studies have tried to understand how bacteriocin production is regulated. This is frequently done through quorum sensing and as part of stress responses to DNA damage, oxidative stress, membrane envelope stress, nutrient limitation and abiotic stress, which can be informative cues as to how effective it would be to produce toxins [8, 18]. For example, studies frequently induce bacteriocin production by addition of DNA damaging agents such as Mitomycin C [16, 17, 41]. While many cues such as DNA damage, small signaling molecules and physical stress have been identified, there is still limited understanding regarding their role in natural microbial ecosystems.

Czárán Hoekstra developed a model of toxin regulation based on quorum sensing (QS) [9]. In the model, by detecting local concentrations of other toxin producing cells, individuals can express toxin only when there are sufficient numbers nearby to affect susceptible cells, surpassing a toxin efficiency threshold. Cells could have combinations of toxin production, resistance, QS signal production and detection, each associated with different metabolic costs. In simulations of a mean-field model, the QS system was never selected, even when high toxin concentrations were necessary for effectiveness against susceptible cells. Surprisingly, even in spatially structured cellular automaton environments, QS did not readily evolve and fixate in toxin producers. This was attributed to "cheating" by resistant strains that induce toxin production of others without contributing with allelopathy. QS could only be selected if it was restricted to toxin producing strains, and still required low costs and intermediate toxin efficiency thresholds.

This illustrates that understanding advantages of spite regulation based on quorum sensing is not so straightforward. Signaling and regulatory systems can be hijacked and tricked by competing strains, which leads to additional complexity in evolutionary microbial warfare at a level analogous to military intelligence and counterintelligence [8, 18, 20]. Although it would be interesting to study the impact of more complex allelopathy regulatory systems, modeling different strategies for toxin regulation was beyond the scope of this project.

4.5 Cheaters - Non producing resistant strains

In our model, we implemented only toxin producing and sensitive strains. In natural populations however, it is common for toxin resistant strains to emerge. These do not contribute with toxin production but can still obtain competitive benefits as sensitives are cleared out, and are frequently referred to as "cheaters". Including resistant strains can lead to cyclic dominance or "rock-paper-scissors" dynamics [10, 16, 30].

Gerardin et al. (2016) performed competition experiments between *E. coli* susceptible, resistant (cheater) and antibiotic producing strains, and these were also reproduced in a computational model [16]. Producer colonies generated inhibition zones that excluded sensitives but were occupied by resistant cells. Producers had the highest selection coefficients when seeded in low starting frequencies with a large density of susceptible cells, but this was decreased by increasing the density of cheaters. Resistant strains had the highest advantage when close to producer colonies and with higher densities of both sensitive and producer cells.

Notably, the largest benefit to producers occurred in intermediate toxin investment and toxin effective ranges. When the toxin becomes too efficient, cheater cells readily occupy inhibition zones compromising advantages to producers. This prediction was validated experimentally by inducing colicin expression and larger inhibition zones with mitomycin C [16] .

Interesting dynamics can emerge in models with toxin-producing, sensitive and resistant strains. Although this was outside the scope of this project, it is a natural extension for further study. As we will see in the next section, however, introduction of additional strains does not necessarily allow complex interactions to be sustained if genotypic competitive exclusion occurs.

4.6 Spatial Structure and biodiversity

Many experiments and models have shown that spatial structure can benefit toxin production by maintaining spatial relationships and increasing local toxin concentrations. Spatial structuring also allows for environment heterogeneity and is important to maintain biodiversity.

In Durrett Levin (1997), a three species model was introduced with a sensitive strain and two immune colicin producers which differed in the amount of toxin production and their growth rates [10]. The second colicinogenic strain can be considered a "cheater" that has a higher growth rate, produces much less toxin but is nevertheless immune. In a well mixed ODE model, the system always flows to a single strain competitively excluding the others. However, in a spatial cell automaton model strains could coexist in the field.

Similar simulation results were obtained by Kerr et al. (2002) in another cell automaton model [30]. The group also performed experiments with colicinogenic, sensitive and resistant *E. coli* strains. When grown in well-mixed flasks or solid plates with thorough colony mixing in-between transfers, diversity could not be maintained. The sensitive strain was readily eliminated, and then resistant strain out-competed colicinogenic bacteria. Only when grown in a static agar plate, all three strains coexisted in the cultures.

In Massey et al. (2004), Asy and X3 strains could only co-inhabit insect infections because caterpillar tissues are spatially structured [33]. When competing Asy and X3 in well-mixed *in vitro* environments, Asy competitively excluded X3, but when grown in static agar plates, both strains coexisted occupying different regions and creating inhibition zones between them. This demonstrates that spatial structuring is important for coexistence of allelopathic strains.

In 1994, Frank performed simulations with a 1-dimensional spatially heterogeneous model, varying the carrying capacity over a line with 3 sequential peaks [13]. Individuals were initially placed in the middle peak and subsequently reproduced and diffused through the field. Interestingly, producers and sensitives could coexist in the field occupying different niches. This was never observed in ODE models, but depended critically on the initial species abundances, the relative carrying capacity and physical width of the peaks. The dimensionless toxin cost, toxicity, diffusivity and decay rate also determined who had an advantage in different niches. He concluded that spatial polymorphism cannot be maintained in well-mixed environments, but can be promoted by spatial variation of environment quality.

Natural microbial communities often form biofilm structures, which can have protective functions and host a large diversity of strains [35]. Spatial structuring in biofilms can have dramatic effects in bacterial interactions, allowing spatial genotype segregation and interfaces between strains in different environments.

Bucci et al. (2011) studied a biofilm structured model of bacteriocin production [4]. Simulations started by inoculating toxin producing and sensitive strains, with biofilm growth limited by a nutrient N. Interestingly, in their model they could derive a critical bacteriocin range that determined at what distance toxins could exclude sensitive cells. This depended on toxicity, toxin diffusivity and production relative to biomass. As expected, increasing the critical bacteriocin range led to competitive advantages to producers in the biofilm, which could be achieved by many parameter combinations. If the critical range was large enough, large investments in bacteriocin production could be sustained. The authors additionally show that intermediate starting frequencies and intermediate investment in toxin production led to higher competitive advantages to producers.

The group studied the influence of nutrient penetration δ, which depended on nutrient concentrations, diffusion rates, bacterial density and thickness of the biofilm. Nutrient penetration indirectly determined the scale of competition. High values of δ lead to relatively homogeneous nutrient concentrations, allow lineage mixing and promote local competition, as focal cells affect mainly their immediate neighbors. Meanwhile, low values of δ promote spatial genetic segregation in the biofilm and global competition, as nutrient uptake limits availability for cells further away. Producers were generally favored with high nutrient penetration and intermediate investment in toxin production. Biofilm structures allowed for coexistence of producer and sensitive strains, but this was strongly influenced by nutrient penetration δ and the critical bacteriocin range. As high values of δ lead to lineage mixing, one strain generally dominates and diversity is lost, especially if the range of bacteriocin effectiveness is larger.

Weber et al. (2014) also studied biodiversity in range expansion experiments and computational models [44]. Growth rates of *E. coli* resistant, sensitive and colicinogenic strains were experimentally manipulated to obtain cyclic rock-paper-scissors dominance. Cyclic dominance alone was not sufficient for coexistence in experimental range expansion fronts, and resistant cells dominated alone or with some colicinogenic regions.

Estimating parameters from experiments, the authors developed a model that could reproduce the behavior of colony range expansion. Broad parameter sweeps were explored in the model measuring the front biodiversity, allowing "biodiversity laws" to be derived. Generally, biodiversity could be maintained by (1) lowering the frequency of colicinogenic strains and increasing resistant strains, (2) lowering the toxin effective range while increasing colicinogenic frequency and (3) balancing the relative growth rates of the strains. These results show that diversity can only be sustained with a balance of initial strain frequencies, relative growth rates and toxin effectiveness, jointly determining the success of each strain in range expansions.

4.7 Model limitations

Naturally, all modeling work involves assumptions and limitations. It is important to keep in mind that while design choices were biologically inspired by spiteful bacteriocin production, our model is a

simple mathematical abstraction and does not realistically represent features of any particular bacterial community. In this section we briefly comment on some assumptions and modeling choices:

Our implementation of the model synchronized diffusion, growth and death events in the population. Notably, the order of these events can significantly affect simulation results. For example, if opportunities for growth occur immediately after clearance of individuals that have died, toxin producers are disproportionately benefited from decreased competition. In the stepwise model, this allowed much higher levels of spite to evolve. In an attempt to converge to the intended continuous time dynamics, small time steps were taken and diffusion was divided in two substeps in between the growth and death events. An option to avoid artifacts from synchronous discrete events would be to use a Gillespie algorithm. In this case, actions for each individual would be chosen probabilistically, although this can become prohibitively expensive.

Our model assumes that the rate of toxin decay is much faster than the rate of growth. Spite densities ρ_s are only calculated during the death step, and toxins are cleared immediately after. By slowing the toxin decay rate, inhibition zones could linger in the field for longer affecting multiple death steps, which should benefit spite producing strains especially when at low frequencies.

The competition for resources was modeled with a Gaussian competition kernel. This assumes an empty environment is spatially homogeneous with regard to nutrient availability, and the individual competition density ρ_c limits available resources for growth of neighboring individuals. Another option is to model resources explicitly, with spatial coordinates, distinct uptake and turnover rates. This could lead to more realistic models, especially when modeling spiteful behaviors outside of microbial communities.

Spiteful interactions were modeled by increasing the death rate of recipients depending on the distance to toxin producers and the Gaussian spite kernel. Another common approach is to reduce the growth rate of sensitive individuals and assume homogeneous death rates. Results should be qualitatively similar if the growth reduction parameter is chosen to have a similar relative impact as increased death rates.

By using a Gaussian kernel for density estimates, technically all individuals interact with each other in the field, so it is not straightforward to isolate the impact of single individual interactions. However, as the distance becomes larger, the influence of the interaction becomes negligible, which could let us set a cutoff based on the number of standard deviations. Another option would be to use a boxcar kernel function, where a fixed distance determines whether individuals interact.

We have assumed there is no interaction between the metabolic cost of toxin production and nutrient availability. If however, the same nutrients that are limited due to competition are directly used for toxin production, this could increase the relative metabolic cost [13, 46]. It would also be possible to implement the cost of toxin production as a function of the competition density.

Our well-mixed models where $\sigma_d \to \infty$ only homogenize individuals. While they are placed in random positions in each diffusion step, they still have local interactions for growth and spite determined by the standard deviation of the Gaussian kernels. Therefore, although spatial relationships between individuals are destroyed, our well mixed models do not have homogeneous toxin concentrations. Fully well-mixed systems do not require modeling of explicit space and can be described through the use of ordinary differential equations (ODEs). This would allow us to study them analytically and derive phase-planes for all parameters without running expensive simulations [10, 13].

5 CONCLUSIONS

These results provide a comprehensive study on a minimal model of spite selection, investigating effects of the spatial scales of interaction, producer frequency, field densities, metabolic costs and toxin resistance over broad parameter sweeps. We conclude toxin producers benefit from local competition, large spite ranges and limited dispersal. Additionally, we show that the selection of producers also depends critically on the balance of spite expression, toxin effectiveness, initial frequency, and field density. Explicitly modeling scales of interaction for competition, spite and dispersion is a relevant contribution to theoretical studies of allelopathy, and future work could extend this to include complex spatial patterning, spite regulation or strain biodiversity. A thorough understanding of what conditions promote the selection of toxin producers can provide insight on evolution with potential applications in industry and disease.

6 CODE AND DATA AVAILABILITY

Data required to generate the figures, the code for the model and auxiliary scripts are all available at https://github.com/1PTan/spite-SB-internship. The codes for model simulations are written as python 3.8 scripts and figures were created using R version 3.6.2.

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