HELPING AND HARMING

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ABSTRACT

After Darwin (1858) presented the idea of evolution by natural selection scientists focused on the idea of fitness as the personal reproductive success of an individual. Hamilton (1964) gave birth to social evolution by defining what fitness means for a social trait, such as helping another individual by raising their young or harming them by stealing their food. In this thesis we look at the two most unintuitive forms of social behaviour: altruism and spite. In both an individual sacrifices its own personal fitness to help (altruism) or harm (spite) another individual. In my chapters I cover: (i) how the altruistic production of virulence factors in a pathogen can lead to strong frequency and density dependent effects when they are under a volunteer's dilemma, (ii) how these strong effects can lead to year-to-year fluctuations in bacterial virulence and the risk of epidemics, (iii) an argument against indiscriminate spite being possible in finite populations and argue that some previous examples are better classified as selfish indiscriminate harming, and (iv) how spiteful interactions between symbionts can lead to a host evolving higher relatedness amongst the symbionts and eventually result in close mutualisms between the symbiont and the host.

PUBLICATIONS

Chapter 2 is a published paper, "Crystal Toxins and the Volunteer 's Dilemmain Bacteria". Chapter 4 "Can Natural Selection Favour Indiscriminate Spite" is in review at Evolution Letters. Chapters 3 and 5 of my thesis is intended for publication after further formatting. In addition during my DPhil I contributed to a paper related to work done during my Masters, "Uncovering the Rules of Micro-bial Community Invasions".

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Vila, Jean C. C., Matt L. Jones, Matishalin Patel, Tom Bell, and James Rosindell (Aug. 2019). "Uncovering the Rules of Microbial Community Invasions." In: *Nature Ecology & Evolution* 3.8, pp. 1162–1171. ISSN: 2397-334X. DOI: 10.1038/s41559-019-0952-9.

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CONTENTS

1

INTRODUCTION

1.1 INTRODUCTION

Helping and harming is rife within the tree of life. Most organisms interact with members of their own species throughout their lifecycle. The fitness consequences of these interactions can have a large effect on an organism's fitness. Even superficially asocial traits such as a solitary predator's hunting behaviour can have social consequences, to the predator's offspring, for example, or its rivals in adjacent territories. This social nature to evolution was understood in some sense by early evolutionary biologists, however the focus was always on the reproductive success of an individual (Darwin, 1858; Fisher, 1930). It was Hamilton (1964) who first conceptualised how traits could evolve that reduced the reproductive success of an individual but benefited its social peers.

Hamilton (1964) had the key insight that when a gene influences the fitness of another individual it has some chance of influencing its own representation in the next generation. A gene for parental care for example could act to increase the fitness of an individual's offspring, and for a sexual diploid there would be a probability of 0.5 (relatedness) that the offspring would also contain that gene for parental care. This statistical association

extends not only to direct kin however but also to the entire population (Grafen, 1985; Queller, 1992; Pepper, 2000). Hamilton defined inclusive fitness as a way to understand these effects. The inclusive fitness of an individual is the sum of: the fitness of that individual, stripped of all help or harm from others, and the effect on fitness of the individual on each other individual in the population (including itself) weighted by the relatedness to that other individual (Hamilton, 1964, 1970).

The question Hamilton sought an answer to using inclusive fitness was, When will an organism sacrifice its own reproductive success to increase the success of another individual? When will we see altruism evolve? Hamilton's rule is the condition he found, $RB - C > 0$. Where R is the relatedness between two individuals, B is the benefit the focal individual (actor) gives to another (recipient), C is the cost that actor pays to perform the behaviour.

Hamilton's rule allows us to categorise all social interaction into four main groups: (i) altruistic behaviours, with a positive cost and benefit, (ii) spiteful behaviours, with a positive cost and a negative benefit, (iii) mutualistic behaviours, with a negative cost and a positive benefit, (iv) selfish behaviours, with a negative cost and a negative benefit (table 1.1).

Classification	Cost	Benefit
Altruistic	$+C$	$+B$
Spiteful	$+C$	$-B$
Mutualistic	$-C$	$+B$
Selfish	$-C$	$-B$

Table 1.1: The sign of both the cost and the benefits classifies all social interactions into one of four groups.

1.2 HELPING AND HARMING

Arguably the most interesting traits in this four-way classification are altruism and spite. Mutualisms and selfish behaviours are easily justifiable because they lead to direct increase in the reproductive success of the individual. Altruism and spite however require the loss of fitness, which from a purely classical view of fitness makes little sense and only seems plausible once we consider it through the lens of inclusive fitness.

1.2.1 *Altruism*

In chapter 2 I focus on modelling a bacterial example of altruism. Social behaviours in general and altruism especially appears common amongst microbes (Crespi, 2001; Stuart A. West et al., 2007). Bacteria form clonal colonies that can have high levels of relatedness amongst individuals. They also perform a wide range of extracellular behaviour by releasing proteins and other molecules into the environment. This means many bacterial traits have social consequences (Stuart A. West et al., 2006, 2007). This range of examples and mechanisms make bacteria attractive to study both experimentally and theoretically.

Bacteria often engage in behaviours that lead to production of public goods. Because, factors are secreted into the environment any nearby bacteria can try to take advantage of the beneficial products of another. This makes many public goods social as they benefit nearby bacteria as well as the individual. The reason

this can be selected for is either the personal benefit is high enough, the trait is selfish, or those benefiting are highly related to the individual producing the good and therefore the trait is altruistic.

1.2.2 *Spite*

In chapter 4 I consider whether indiscriminate spite can evolve. Spite using Hamilton's rule is expressed as a positive cost and a negative benefit:

$$\begin{aligned} R(-B) - (+C) &> 0 \\ -RB &> C\,. \end{aligned} \tag{1.1}$$

To spread in the population then spite requires a negative relatedness between an actor and a recipient. There are two issues with this fact: (1) What does negative relatedness even mean?, (2) how can it arise? Firstly, relatedness is measured with respect to the population average (Grafen, 1985; Pepper, 2000). This means an individual with the average relatedness to the focal individual is considered related to that individual with a coefficient of 0 even if the absolute chance that they share a gene is non-zero. Secondly, if an individual can target individuals it knows are related to it less than the population average then their relatedness is negative with respect to the population average. This requires the actor to collect information about the relatedness status of the recipient, kin discrimination (A. Gardner and S. A. West,

2004; Andy Gardner and Stuart A. West, 2004; Andy Gardner et al., 2007). Indiscriminate spite is where the harming behaviour happens regardless of the relatedness between the actor and the recipients. I show how results in support of indiscriminate spite should be more accurately classified as selfish indiscriminate harm.

1.3 SUMMARY OF THESIS

In my thesis I develop evolutionary theory to understand helping and harming behaviours in the natural world. Oftentimes the help and harm an individual does can have knock-on second order effects to members of its own species and to members of other species. These feedbacks complicate the analysis of social traits. Rather than ignore these effects I have attempted to add back some elements of these dynamics to see if it can better predict the patterns seen in nature.

CHAPTER 2 — In this chapter I explore the effects of a volunteer's dilemma on the maintenance and success of a parasitic bacterium. In the volunteer's dilemma only a subset of the group has to produce a public good to benefit all members equally. This causes a large payoff advantage of defection. I investigate this using the production of Crystal toxins by the insect pathogen *Bacillus thuringiensis*.

Contribution: The idea was developed between Patel, Raymond and West. All modelling was done by Patel. The final

manuscript was written by Patel with thoughts and comments by Raymond, West and Bonsall.

CHAPTER 3 — Following from Chapter 1, I derive a set of difference equations for the growth of *Bacillus thuringiensis* populations. Using these equations I try to find patterns of strategy variance in the bacterial population dynamics which are thought to occur in nature.

Contribution: The idea was developed between Patel and West. All modelling and writing was done by Patel with comments and advice from West and Bonsall.

CHAPTER 4 — It is understood that spite can evolve when individuals discriminate between kin and non-kin. However, some authors have argued that indiscriminate spite can also evolve. In this chapter I propose that previous work on indiscriminate spite has misclassified the behaviour due to an incorrect partitioning of fitness effects. I derive a partitioning that makes a finer distinction between direct and indirect effects and show that previous theory on spite may have actually been describing a selfish behaviour. Indiscriminate spite may not evolve but indiscriminate harming may.

Contribution: The idea was developed between Patel and Biernaskie. Initial modelling and writing was done by Patel. Final draft was contributed to by Biernaskie and West.

CHAPTER 5 — The evolution of symbiosis is one of the most extreme forms of cross species helping. It has been shown

that increasing relatedness amongst symbionts within a host leads to higher levels of cooperation between the host and the symbiont. However, Frank (1996) identified that a mutant host that increased relatedness amongst its symbionts would not gain an advantage until many generations down the line. Therefore, an immediate advantage to increasing relatedness must exist. I present a model for symbiosis where that immediate advantage is the reduction in bacteriocin production amongst symbionts. This increases the productivity of bacterial symbionts in the short term and in the long term can lead to higher levels of cooperation with the host. I present an illustrative model and a set of individual based simulations to support these ideas.

Contribution: The idea was developed between Patel and West. All modelling and writing was done by Patel with comments and advice from West.

CHAPTER 6 — I discuss the work presented in my thesis. I identify key areas highlighted by my work that merit further exploration and clarification.

I have kept this introduction short as this is submitted as an integrated thesis, and as such each chapter has its own comprehensive introduction.

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2

CRYSTAL TOXINS AND THE VOLUNTEER'S DILEMMA

Crystal Toxins and the volunteer's dilemma in bacteria.

Matishalin Patel, Ben Raymond, Michael B. Bonsall, Stuart A. West

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1 Abstract

The growth and virulence of the bacterium *Bacillus thuringiensis* depends on the production of Cry toxins, which are used to perforate the gut of its host. Successful invasion of the host relies on producing a threshold amount of toxin, after which there is no benefit from producing more toxin. Consequently, the production of Cry toxin appears to be a different type of social problem compared with the public goods scenarios that bacteria usually encounter. We show that selection for toxin production is a volunteer's dilemma. We make specific predictions that: (1) selection for toxin production depends upon an interplay between the number of bacterial cells that each host ingests, and the genetic relatedness between those cells; (2) cheats that do not produce toxin gain an advantage when at low frequencies, and at high bacterial density, allowing them to be maintained in a population alongside toxin producing cells. More generally, our results emphasise the diversity of the social games that bacteria play.

keywords:

virulence, cooperation, game theory, kin-selection, evolution, social evolution

2 Introduction

The growth and virulence of many bacteria depends upon successfully cooperating in public goods games with other bacteria. Bacteria produce and secrete a range of molecules, which provide a benefit to the local group of cells, and so act as public goods, for example, iron scavenging siderophores, or protein digesting proteases (West, Diggle, Buckling, Gardner and Griffin, 2007). Individual cells pay the metabolic cost of producing these molecules, but their benefits are then shared as public goods with the local population of cells. Consequently, producing cells could potentially be out-competed by non-producing, cheats, who gain the benefits, without paying the costs. There is a large theoretical and empirical literature examining how various factors such as interactions between genetically identical cells (kin selection), can stabilise the production of public goods in bacteria (Brown and Johnstone, 2001; West and Buckling, 2003; Griffin et al., 2004; Diggle et al., 2007; Frank, 2010*b*,*a*).

In contrast, the growth and virulence of the bacterium *Bacillus thuringiensis* appears to depend upon a different type of social game (Raymond et al., 2012). The life cycle of this bacterium depends upon two steps in the host. First, after an insect host ingests a number of spores, the bacterial cells use a costly crystal (Cry) toxin to perforate the host gut, and invade the host (Höfte and Whiteley, 1989; Ibrahim et al., 2010; Raymond et al., 2012). The toxin is a large protein, up to 147 kilodaltons, that may form up to 35% of a bacterium's dry mass (Loferer-Krößbacher et al., 1998). Second, the bacteria multiply within the host and invest in Cry toxin production, causing host

death and the release of bacterial spores (Raymond et al., 2010). In contrast to a public goods scenario the benefit of producing Cry toxin is relatively all or nothing — you either produce enough to invade the host, or you do not. As producing a certain amount of total toxin is key, the strategy that will be favoured by evolution could also depend upon the number of spores that are inside a host (Archetti, 2009; Raymond and Bonsall, 2013; Cornforth et al., 2015).

We examine the evolutionary stability and dynamics of Cry toxin production using two different modelling approaches. First, we use a game theoretic approach to examine under what conditions the production of Cry toxin is favoured (Taylor and Frank, 1996). This approach assumes only small variations in toxin production (weak selection), and looks for a single equilibrium. In contrast, in nature there is large variation in toxin production, between cells that produce (cooperators) or do not produce (cheats) Cry toxin (Raymond et al., 2010, 2012). Deng et al. (2015) find that cheaters produce on average 30% more spores than cooperators. This variation is driven by both differences in copy number of Cry toxin genes and the presence or absence of a plasmid carrying the genes (Höfte and Whiteley, 1989; Raymond and Bonsall, 2013). This led us to consider both a weak selection model (mutations to Cry genes) and a strong selection model (gain or loss of a plasmid carrying the Cry genes). Furthermore, factors such as population density and cooperator frequency can fluctuate over short timescales (Schoener, 2011; Raymond et al., 2012; Gokhale and Hauert, 2016), and studies of the density of spores in the wild have shown that group sizes are very low suggesting that stochastic effects could be important (Madudell et al., 2002; Collier et al., 2005; Raymond et al., 2010). Therefore, our second approach is to model the dynamics of a system that contains both co-operators and cheats, to examine how these dynamics are influenced by bacterial density, and the frequency of cooperators.

3 Model I: Equilibrium Model

We use a game theoretic approach to express the fitness of a bacterial cell as a function of: the probability it infects a host, $\beta(z)$; and, the number of spores it generates, $f(y)$ — where, z is the group average strategy and y is the individual cells strategy. We assume an infinitely sized population of bacteria distributed into finitely sized patches of n bacteria. There are non-overlapping generations and the bacterial spores disperse randomly to other patches.

We assume that the probability that a bacterium in a group of n cells successfully infects a host, β, is a function of their average investment, z. We model this probability using a sigmoidal curve as a continuously differentiable approximation of a step function:

$$\beta(z) = \frac{1}{1 + e^{-(nz-k)}}, \tag{1}$$

where, the group production of toxin nz is compared to k, which is the threshold at which the chance of infection would be 0.5 (Cornforth et al., 2012). When the total toxin production is low ($nz << k$) then the chance of infection is close to 0 as toxin production increases $0 \leq nz \leq k$ then the function is accelerating and then past the threshold ($k < nz$) the function is decelerating and asymptotes to 1.

We assume there is a linear trade-off between the energy a bacterium puts into producing toxin, y, and the energy available for growth, f — as both require the generation of protein:

$$f(y) = 1 - ay, \tag{2}$$

where a is the cost per unit of toxin and the baseline fecundity is 1. The fitness function of a focal bacterium will be the product of the probability

it invades a host and the growth of the bacterium once it has successfully invaded ($\beta(z) \cdot f(y)$):

$$\omega(y,z) = \frac{1-ay}{1+e^{-(nz-k)}}. \tag{3}$$

Equation (3) illustrates that producing the Cry toxin has a cost to the individual by reducing its growth, $f(y)$. However, it is beneficial to the group, including our focal individual, as it increases the chance of successful invasion, $\beta(z)$. The production of Cry toxin is either mutually beneficial or altruistic depending on parameter values (Rousset, 2004; West, Griffin and Gardner, 2007). This is a similar but altered formulation to Archetti and Scheuring (2011) as we use a multiplicative rather than additive cost. Having a multiplicative cost retains meaningful dimensions for the fitness by multiplying a probability by a relative fecundity, which allows an interpretation for fitness (ω) as the actual fecundity expected by an individual.

We seek an evolutionarily stable strategy (ESS), which is the individual strategy at fixation which cannot be invaded by some rare alternative strategy. Following Taylor and Frank (1996), we construct an expression for the change in inclusive fitness, $\Delta\omega_{IF}$, and solve for a monomorphic population that is at equilibrium:

$$\begin{aligned} \Delta\omega_{IF}\Big|_{y=z=z^*} &= 0 \\ z^* &= \frac{1}{a} - \frac{1}{r(n-1)+1} - \frac{W(a,k,n,r)}{n}, \end{aligned} \tag{4}$$

where, W is a Lambert-W function which is strictly positive (see B) and r is the relatedness between the different bacterial cells infecting the host. We define r as the probability that two individuals share the same gene at a locus relative to the population average (Grafen, 1985). This measure is obtained by replacing the regression of the recipients phenotype on the

focal individuals genotype (R in Taylor and Frank (1996)) with: $R = \frac{1}{n} + \frac{n-1}{n} r$. Where $1/n$ is the chance the other individual is oneself and $n - 1/n$ is the chance of a social partner with other's only relatedness r to the focal individual (Pepper, 2000).

The equilibrium at z^* is a maximum however it may be unreachable. To test whether a population under weak selection would converge to equilibrium (convergence stability), we examined whether the second order terms at the equilibrium were negative (Otto and Day, 2011). We found that the derivative of the change in inclusive fitness with respect to y was less than 0 at equilibrium:

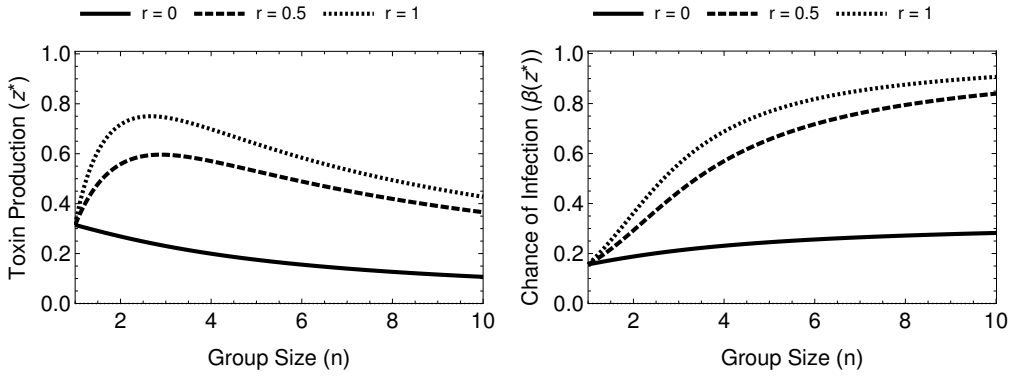
$$\Delta\omega'_{IF}\Big|_{y=z=z^*} < 0 \tag{5}$$
$$\text{if:} \quad a > 0,\ 0 \leq r \leq 1,\ n \geq 1, \text{ and } W \geq 0.$$

So the equilibrium at z^* is a candidate ESS. To determine uninvadibility we implement an extension to the Taylor and Frank (1996) approach, by interpreting the second derivative of the fitness equation in terms of inclusive fitness effects, therefore establishing a condition for the candidate equilibrium to be a local maximum (Cooper and West, 2018). In appendix A we show that z^* is an uninvaidable strategy as well as being convergently stable.

3.1 The effect of relatedness

We found that increasing relatedness (r) increases individual toxin production. Examining the derivative of the equilibrium toxin production (z^*) with respect to relatedness (r) we found that:

$$\frac{\partial z^*}{\partial r} \geq 0 \qquad \forall r; r \in [0,1]. \tag{6}$$



(a) (b)

Figure 1: The equilibrium toxin production depends on group size (n) and relatedness (r). **a)** When $r > 0$, as we increase group size, toxin production initially increases and then decreases. **b)** The total amount of toxin produced by the group, nz^*, increases with group size, therefore, the chance of infecting the host is always higher in larger groups (appendix E). These graphs assume $k = 2$ and $a = \frac{2}{3}$ (appendix D).

So as relatedness (r) within the group increases the ESS of toxin also increases (z^*) (appendix C). Increasing relatedness increases the indirect benefit from toxin production as the group chance of invasion, $\beta(z)$, has a greater chance of being shared with kin. However, even when relatedness is low ($r = 0$) toxin production is favoured as it is essential to reproductive success (fig. 1).

3.2 The effect of group size

As groups increase in size individual toxin production initially peaks and then declines — when relatedness is non-zero (fig. 1a). This is due to the efficiency gained when close to the accelerating section of the sigmoidal $\beta(z)$ function (near the threshold). As the benefits ($\beta(z)$) are accelerating, small increases in toxin production lead to large increases in infection chance. Past the peak toxin production, the greater number of individuals in the patch allow for individual bacteria to reduce their investment but the group remains at a high chance of successfully invading (see appendix E).

3.3 The effect of the threshold

The derivative of toxin production, z^*, with respect to the threshold is always positive or zero:

$$\frac{\partial z^*}{\partial k} = \frac{W\left(\frac{ne^{n\left(\frac{1}{a}-\frac{1}{r(n-1)+1}\right)-k}}{(n-1)r+1}\right)}{n\left(W\left(\frac{ne^{n\left(\frac{1}{a}-\frac{1}{r(n-1)+1}\right)-k}}{(n-1)r+1}\right)+1\right)}$$

$$\frac{\partial z^*}{\partial k} > 0 \quad \text{if}: a > 0, k \geq 0, n \geq 1, 0 \leq r \leq 1. \tag{7}$$

Therefore, if more toxin is required to invade the host (higher k) individuals will be selected to increase their toxin production (z^*).

3.4 Cry toxin as a Volunteer's Dilemma

Our model illustrates that the production of crystal toxin by the bacteria is a volunteer's dilemma. Volunteer's dilemmas are a class of social games where the benefit is gained after a threshold investment in the good is reached and the benefit is fixed for each member regardless of group size or personal investment (Archetti, 2009, 2018). The perforation caused by Cry toxin can be used by any organisms in the gut, it is a public good. The Cry toxin only perforates the gut after a certain concentration, the good acts after a threshold (eq. (1)). And, the benefit to the bacteria is access to the tissue of the host which is a binary outcome, either the bacteria have access or not, there is no additional benefit for exceeding the threshold of Cry toxin in the gut (Höfte and Whiteley, 1989; Ibrahim et al., 2010; Raymond et al., 2012). These qualities of the Cry toxin system make its production a volunteer's dilemma.

4 Model II: Cooperator-Cheat Dynamics

In nature the density and fraction of spores, that either do (cooperators) or don't (cheats) produce Cry toxins, can be very variable over short temporal and spatial scales (Maduell et al., 2002; Collier et al., 2005; Raymond and Bonsall, 2013). We capture this ecological variation with a model which allows us to compare individuals that produce toxin at a fixed level (cooperator) against individuals which do not produce any toxin (cheats). We compare the relative fitness between these two types to determine under varying ecological parameters.

We assume a population of bacteria whose spores freely mix and are taken up at random by a host. We assume that the host ingests P bacterial spores. In the environment a proportion (c) of bacteria are cooperators and $(1-c)$ are cheats. For a focal individual in a group of $P-1$ social partners there are i cooperators which are distributed:

$$Pr(i) \sim \text{Binomial}(P-1, c) = \binom{P-1}{i} c^i \, (1-c)^{P-1-i}. \tag{8}$$

From eq. (3) given i cooperators in a group the payoff, π, for the focal bacterium producing y toxin will be:

$$\pi_i(y,z) \; = \; \frac{1-ay}{1+e^{-(iz+y-k)}}. \tag{9}$$

Therefore, the overall fitness of a focal bacterium producing y toxin in a population of cooperators producing z toxin will be:

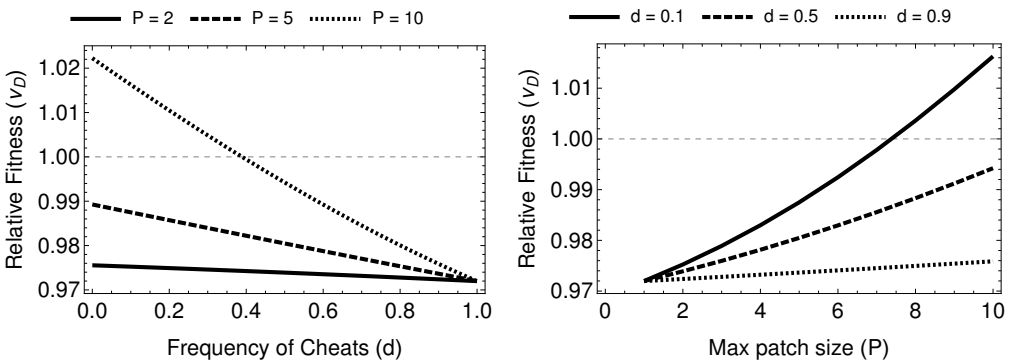
$$\omega(y,z) = \sum_{i=0}^{P-1} \binom{P-1}{i} c^i \, (1-c)^{P-1-i} \, \frac{1-ay}{1+e^{-(iz+y-k)}}. \tag{10}$$

This allows us to express the fitness of a cooperator in the population as $\omega(z,z)$ and that of a cheat as $\omega(0,z)$. The relative fitness of cheats to cooper-

ators in the population is:

$$\nu_D = \frac{\omega(0,z)}{\omega(z,z)} \tag{11}$$

This model assumes a large trait difference between cooperators and cheaters (strong selection).



(a) (b)

Figure 2: **(a)** The relative fitness of cheats is negatively frequency dependent as cheats become more common they are more often aggregated together and so suffer in relative fitness to cooperators. **(b)** As group size increases there is a positive density dependent effect on cheat fitness, the larger the group the more chance that sufficient toxin is produced by the group. Using parameters: $k = 2$, $a = 2/3$ and $z = 0.17$ (appendix D).

4.1 Frequency dependence

As the proportion of cheats increases we find that the relative fitness of cheats decreases (fig. 2a). As cheats become more common, groups become dominated by cheats and the chance that a group produces enough toxin decreases. Why do we find frequency dependence when, in the simplest possible case, a public goods dilemma leads to selection being frequency independent (Ross-Gillespie et al., 2007).

The result of frequency independence requires either: (1) that the effect on public good production is linear or, (2) that the trait is under weak selection. Either of these two assumptions make a linear approximation, using a first order Taylor expansion, valid. And, such expansions, are frequency independent (Rousset, 2004; Lehmann and Rousset, 2014). This argument

is similar to the justification for frequency independent selection of a trait that the selection gradient, $s(z) = \partial\omega/\partial y + r\partial\omega/\partial z$, is constant with respect to allele frequency (Hamilton, 1964; Gore et al., 2009; Lehmann and Rousset, 2014).

However, in our model we find that the relative fitness of a cheat is frequency dependent. This is because we relax both of the assumptions made by Ross-Gillespie et al. (2007). We have a non-linear synergistic effect between cooperators which means that each cooperator or defector does not have a linear effect on the fitness of the focal individual, due to the step like benefit function ($\beta(z)$). Addition or subtraction of a cooperator has a large effect when a group is close to the the threshold but a much smaller effect when the group toxin production is already very low or very high; the benefit of a cooperator is dependent on the compisition of the group which is itself dependent on the frequency of cooperators. This synergy introduces a frequency dependent term into the first order effects of our selection gradient (Lehmann and Rousset, 2014). Secondly, we consider a game with strong selection which makes approximating the gradient using only first order terms inappropriate. The large difference between cooperator and cheat strategies causes higher order terms of the relative fitness to matter and these higher order terms will include frequency dependent terms (Hamilton, 1964; Ross-Gillespie et al., 2007).

These two effects lead to a frequency dependent relative fitness found here — unlike the frequency independence found in earlier models (Ross-Gillespie et al., 2007). The synergistic game causes the first order term of the Taylor expansion to be frequency dependent. The strong selection causes higher order terms to become more substantial. These two effects are sufficient but not necessary conditions for frequency dependence to arise.

4.2 Density dependence

Increasing the density of the population, by increasing the group size (P) while holding the frequency of cooperators constant, increases relative cheat fitness. Figure 2b shows that in more dense populations there is a greater chance that a group will have a sufficient number of cooperators to invade a host successfully (Ross-Gillespie et al., 2009). The mean number of cooperators in a group increases with density allowing cheats to exploit more cooperators. In the limit, as P increases, the chance of infection for all patches in the population is one, ($\beta(z^*) = 1$). Therefore, the fitness of cheats is 1 and the fitness of all cooperators is $1 - az$. The relative fitness of cheats then approaches, $1/(1 - az)$.

4.3 Population Aggregation

The above model assumes patches form randomly from the population with no structuring beyond random chance. We now imagine a scenario where similar strategies are clumped together, as would be expected if they had emerged from the same host (van Leeuwen et al., 2015). We use a modified Poisson binomial distribution to model the initial member of a group biasing subsequent draws towards its own type. As initial founders are randomly distributed, a fraction c of the groups are clumped around a cooperator and a fraction d around a cheat ($c + d = 1$).

So given that the patch is started by a cooperator then the of number of cooperators among such patches is given by the probability mass function:

$$\underbrace{\binom{P-1}{i-1}}_{\text{less founder}} \quad \underbrace{(c+\delta_1)^{i-1}}_{\text{Pr (i cooperators)}} \quad \underbrace{(1-(c+\delta_1))^{P-i}}_{\text{Pr (P-i cheats)}}, \tag{12}$$

and similarly for cheats:

$$\underbrace{\binom{P-1}{i}}_{\text{less founder}} \quad \underbrace{(1-(d+\delta_2))^i}_{\text{Pr (i cooperators)}} \underbrace{(d+\delta_2)^{P-i-1}}_{\text{Pr (P-i-1 cheats)}}. \tag{13}$$

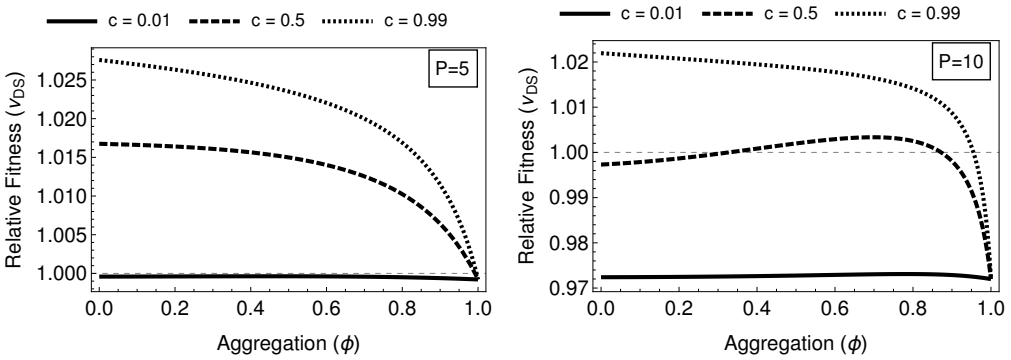
The binomial coefficient is $C(P-1, i-1)$ for cooperators as the founder individual counts for the first group member and the first cooperator. For the defector patches the founder only accounts for the first group member, hence $C(P-1, i)$. The two variables, δ_1 and δ_2, are terms that bias the distributions based on the founder. The larger their values the more strongly the two types aggregate. These two distributions represent an underlying distribution — that of the simpler model. We define $\phi \in [0,1]$ as the level of aggregation and define the bias parameters as: $\delta_1 = \phi c$; $\delta_2 = \delta_1 \frac{c}{d}$. When ϕ is one then patches of all cooperators and all cheats form and when it is zero then there is no bias and patches form as they would in a binomial distribution. By expressing the bias parameters (δ_1 and δ_2), in terms of ϕ, c and d, we ensure that the sum over both distributions is equal to one, and the terms are weighted probabilities.

The distribution of the number of cooperators in a patch is weighted by the fitness of the focal individual in such a group (the sum of the above two distributions), giving:

$$\omega_S(y,z) = \sum_{i=0}^{P-1} \Bigg(c\binom{P-1}{i-1}(c+\delta_1)^{i-1}(1-(c+\delta_1))^{P-i} \quad + \\ (1-c)\binom{P-1}{i}(1-((1-c)+\delta_2))^i((1-c)+\delta_2)^{P-i-1}\Bigg)\pi_i(x, i\cdot y), \tag{14}$$

and from this we calculate a structured relative fitness: $\nu_{DS} = \omega_S(0,z)/\omega_S(z,z)$

At maximum aggregation ($\phi = 1$) cheats will do very poorly against cooperators as groups formed of all cheats have almost zero chance of invading the host. In the absence of aggregation ($\phi = 0$), cheats will be perform-



(a) (b)

Figure 3: Graphs of ω_S, eq. (14), using parameters: $k = 2$, $a = 2/3$ and $z = 0.17$ (appendix D). **(a)** When group size is low, $P = 5$ increasing aggregation leads to decreasing relative fitness for cheats regardless of the initial cooperator frequency **(b)** At higher group sizes ($P = 10$) the pattern is also decreasing at high cooperator frequencies however at middling and low densities we see a non monotonic pattern with an intermediate aggregation causing a maximum relative fitness in cheats.

ing as if the population were unstructured, as in the previous model. As aggregation increases cheats are more likely to find themselves in groups composed mostly of cooperators or mostly of cheats and very rarely a group close to an unbiased distribution.

Intermediate levels of aggregation can, with intermediate frequencies of cooperators and high densities, lead to an increase in cheat relative fitness (fig. 3). When group sizes are large the benefit to all members of a group of cooperators will approach one. At that point any additional cooperators will perform much worse than additional cheats as they will be paying the cost of producing toxin and gaining no marginal benefit from this additional toxin (infection chance can't be greater than one). Therefore, at intermediate levels of aggregation, enough cooperators will be on patches to infect a host and be exploited by cheats. Conversely in the defector biased groups the threshold will never be reached and cooperators perform poorly as they are paying a cost for little benefit and any generated benefit is being exploited by cheats. This leads to high density scenarios with intermediate levels of aggregation increasing cheat relative fitness.

The above method of looking at the relative fitness of cheats to cooper-

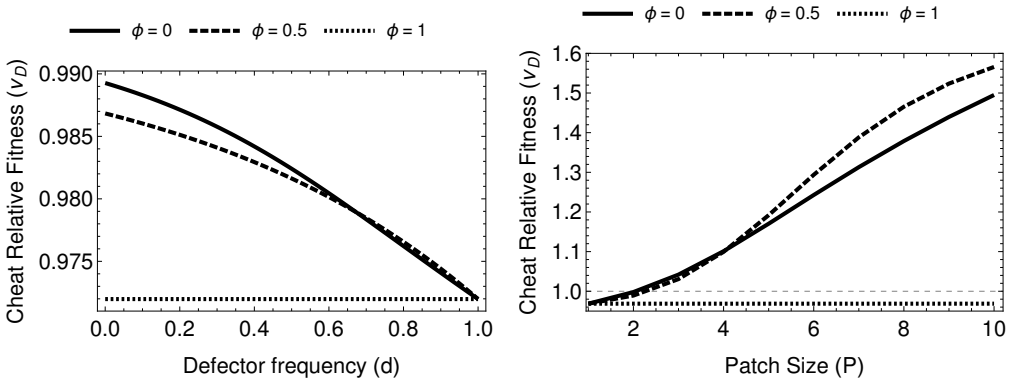


Figure 4: **(a)** Frequency dependence is still present in the base case of no aggregation as aggregation increases a critical point is reached at full aggregation where frequency dependence disappears **(b)** Increasing density increases cheat fitness as long as aggregation is again less than one. At full aggregation the density dependent effect disappears. Using parameters: $k = 2$, $a = 2/3$ and $z = 0.17$ (appendix D).

ator shows whether a cheat will be increasing or decreasing in the population. This gives a static view of the dynamics occurring in the population. Our analysis shows how cheats can have a high enough relative fitness to invade a population and some predictions on what would happen as environmental and demographic parameters change. However, they cannot establish over the long term whether cheating is a stable strategy in a population. In appendix F we show that cheat-cooperator co-existence can be reached dynamically from our model (Peña et al., 2014; Archetti, 2018).

5 Discussion

The production of toxin by the bacterium *B. thuringiensis* is different from the kind of public goods game that is usually imagined in bacteria. The threshold nature of the toxin production leads to a volunteer's dilemma where for each individual it would be optimal if another were to volunteer to produce the good (toxin) instead of them. We found, with a game theory approach, that the ESS level of toxin production: (1) increases when the cells infecting a host host are more related, and (2) peaks at intermediate

numbers of cells infecting a host (fig. 1). We then developed a stochastic model of the dynamics of cooperators that produce toxin, and cheats that do not produce toxin. We found the relative fitness of cheats was greater when: (1) they were less common (lower frequencies), (2) more cells infect each host (higher densities) (fig. 2), (3) cells partially aggregated with the same cell types (relatives) (fig. 3). Our results show how ecological conditions can influence the relative fitness of cheats and cooperators, in ways that could feedback into the population dynamics of *B. thuringiensis* and its invertebrate hosts.

5.1 The volunteer's dilemma for public goods.

The volunteers dilemma is a very widely applicable game. Bacterial signalling pathways that require quorum sensing could fall under the umbrella of volunteer's dilemmas (Darch et al., 2012). Costly signalling in general about environmental conditions is likely to be a volunteer's dilemma. The production of toxins with threshold conditions (such as Cry toxin) are all volunteer's dilemmas. Identifying how non-linear public goods games such as volunteer's dilemmas differ in behaviour from tradition linear public goods games helps us understand a diverse set of games.

Assuming that toxin production resembles a volunteer's dilemma game leads to some different predictions compared with other social traits in bacteria that have been studied (Brown, 1999; Brown and Johnstone, 2001; West and Buckling, 2003; Ross-Gillespie et al., 2007; Ross-Gillespie et al., 2009; Frank, 2010*a*). We found that individual investment (toxin production) is highest at intermediate group sizes, that the fitness of cheats can depend upon their frequency in the population (frequency dependence) in well mixed populations, and that intermediate levels of aggregation can increase the relative fitness of cheats (Archetti, 2009, 2018). In contrast, in linear public goods games, toxin production is not frequency dependent in

well mixed populations, and intermediate levels of aggregation decrease the relative fitness of cheats (Brown and Johnstone, 2001; West and Buckling, 2003; Ross-Gillespie et al., 2007; Ross-Gillespie et al., 2009).

Our result that cheater fitness is dependent upon the frequency in the population contrasts with Hamilton (1964) "gift from god" that cooperator fitness should be independent of frequency. Our analyses differ from Hamilton's in two ways. Firstly, in the volunteer's dilemma, each additional player has a non-linear effect (non-additivity) on the benefit, which means that even when looking at first order terms frequency is present as a variable (Rousset, 2004). Secondly, in our models we assume that the cheater produces no toxin and the cooperator produces a large quantity, leading to strong selection, which means that linearising the relative fitness is no longer appropriate as higher order terms have large effects (Ross-Gillespie et al., 2007; Gore et al., 2009; Lehmann and Rousset, 2014). In nature Cry toxin genes often occur on plasmids so large loss and gain of function mutations are possible, therefore we consider a strong selection model to be more accurate for the natural dynamics (Ibrahim et al., 2010). A more explicit model with a continuous scale of toxin production would extend our model to cover a greater number of biological scenarios.

5.2 Bt in the wild

Our results are supported by both observational and experimental data from field populations of *B. thuringiensis*. Consistent with our prediction that frequency dependent selection can lead to cooperators and cheats coexisting, natural populations show variation in the level of cry toxin production, with both producers and non-producers coexisting (Raymond et al., 2010, 2012; Raymond and Bonsall, 2013). Also, as predicted by our model, experimental manipulations have found that the relative fitness of cheats is higher when they are at lower frequencies in the populations, and at

higher densities (frequency and density dependent selection) (Raymond et al., 2012).

Our model also makes novel testable predictions. We predicted that the fitness of cells that do not produce toxin (cheaters) depends upon an interaction between aggregation and density (fig. 4), and that toxin production should peak at intermediate group sizes (fig. 1). These predictions could be tested with field manipulations or experimental evolution. Our results also suggest the possibility for interactions between evolutionary and ecological (population) dynamics, that require further theoretical and empirical work. For example, low cell densities at the start of a season would favour cells that produce toxin (cooperators), which would lead to an increase in cell densities. This would favour cells that do not produce toxin (cheaters), which could reduce cell densities and now favour toxin producers again. Furthermore, these changes in cell densities and the frequency of toxin producers would also impact on the population dynamics of their invertebrate hosts, which could also influence the number of cells infecting each host (Raymond et al., 2012). These dynamics could potentially lead to seasonal patterns and/or intermittent epidemics of *B. thuringiensis*. The interplay of evolutionary and ecological dynamics between toxin non-producers and toxin producers has previously been demonstrated over the production of an enzyme to beak down sucrose in yeast (Gore et al., 2009; Sanchez and Gore, 2013).

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A Uninvadibility condition

We construct a measure of the change in inclusive fitness caused by changing the focal actors strategy. This is done by expanding the total derivative of the fitness function with respect to a dummy variable for the underlying gene, which yields the expanded derivative (Taylor and Frank, 1996):

$$\frac{d\omega(x,y)}{dg} = \frac{\partial\omega}{\partial x}\frac{dx}{dg} + \frac{\partial\omega}{\partial y}\frac{dy}{dg} \tag{15}$$

Following from Taylor and Frank (1996) we make the substitutions of the phenotypic derivatives for regression coefficients and then simplify to get an expression of change in inclusive fitness:

$$\Delta\omega_{IF} = \frac{\partial\omega}{\partial x}\cdot 1 + \frac{\partial\omega}{\partial y}R \tag{16}$$

In the paper we then analyse the behaviour of $\Delta\omega_{IF}$ and $\Delta\omega'_{IF}$ to characterise the equilibrium as maximal and convergent. Cooper and West (2018) method is used to determine if the equilibrium is unavailable. In brief, we consider the second derivative of the total derivative taken to obtain the inclusive fitness effects (Taylor and Frank, 1996). This expands into a long chain rule where we drop all higher order terms (∂g^2 etc.) as negligible and substitute the regression coefficients as before, leaving us with:

$$\frac{d^2\omega(x,y)}{dg^2} \approx \frac{\partial^2\omega}{\partial x^2} + 2\frac{\partial^2\omega}{\partial x\partial y}R + \frac{\partial^2\omega}{\partial y^2}R^2. \tag{17}$$

When this expression is less than zero we can say the equilibrium found is uninvaidable.

B Sign of the ProductLog

W is a Lambert-W function which is strictly positive. The Lambert-W function or ProductLog function is the inverse of the functions in form Xe^X:

$$Y = Xe^X \leftrightarrow X = W(Y) \tag{18}$$

In this case the function in full is:

$$W(a,n,r,k) = W\left(\frac{ne^{n\left(\frac{1}{a}+\frac{1}{-nr+r-1}\right)-k}}{(n-1)r+1}\right) \tag{19}$$

From the above we can see that — assuming: $a > 0,\ 0 \leq r \leq 1,\ n \geq 1, k \geq 0$ — then the function within the brackets will be positive and therefore the value of the function will be a positive real number.

C Analysis of the effect of relatedness

$$\begin{aligned}\frac{\partial z^*}{\partial r} &= \frac{\partial}{\partial r}\left(\frac{1}{a} + \frac{1}{r-nr-1} - \frac{W(a,n,r,k)}{n}\right)\\ &= -(r-nr-1)^{-2}(1-n) - n^{-1}\frac{\partial}{\partial r}(W(a,n,r,k))\\ &= -(r-nr-1)^{-2}(1-n) - \frac{(n-1)^2(r-1)W(a,n,r,k)}{n(1+(n-1)r)^2(1+W(a,n,r,k))}\end{aligned} \tag{20}$$

The expression obtained in eq. (20) is indeed always greater than or equal to zero forall values of r in the internal $[0,1]$. We can see this by first remembering that the function W is always positive for any parameter set which is biologically reasonable — $a > 0,\ 0 \leq r \leq 1,\ n \geq 1, k \geq 0$. We then see that the first term is positive in the denominator (a squared term) and negative

or zero in the numerator ($1-n$ where $n \geq 1$), negating a negative is of course positive so the first term has positive effect on slope. The second term is also negative in the numerator as $r-1$ is always zero or negative. Again it has a positive effect as it is negated from the slope. Therefore as long as the parameters are biologically reasonable the effect of increasing r is to increase investment in the toxin an individual produces.

D Parameter Choice for Figures

We chose a standard parameter set for our graphs of: $k = 2$, $a = 2/3$, $P = 5$, and $z = 0.17$. The cooperator strategy ($z = 0.17$) is derived from the first model. When the strategy z^* (eq. (4)) is evaluated with: $r = 0$, $k = 2$, $a = 2/3$, and $n = 5$. This yields $z^* = \frac{1}{2} - \frac{1}{5} W(5\sqrt{e}) = 0.175132$, which was rounded down to two decimal places to give a conservative estimate of the bacterium's investment into toxin. The threshold $k = 2$ was chosen to ensure that no one cell could have more than 0.5 probability of entering the host and that the the game was social. The cost was set to 2/3 to represent the fact that the tradeoff was between current and future investment so was not directly linear. Also this discount captures the benefit the organism expects to get from investment now into the toxin. Group size was considered to be 5 as the expected groups are small and at a maximum thought to be around 10 spores. Five spores therefore is the average assuming uniform group sizes as the null.

E Parameter sweep for the effect of group size

In the paper we assume a cost of two-thirds and a threshold of two in all scenarios. This was done so that in the case of two individuals total investment by both is necessary to reach the threshold value in $\beta(z)$. The reason that the cost was set to $\frac{2}{3}$ was to represent the fact that the tradeoff is against future

investment not current investment. In fig. 5 we can see a greater range of parameters which are presented here to show that the patterns found are generally true across a reasonable range of parameter space.

F Gain function and interior rest points

A property of the payoff $\omega(z, y)$ (eq. (10)) is that it is a polynomial in Bernstein form (Peña et al., 2014, 2015). This allows us to draw general conclusions about the shape and behaviour of this function by looking at a simple gain function. In essence we can calculate a_i as the payoff for cooperating when i others cooperate, and b_i as the payoff when defecting and i others cooperate.

$$a_i = \frac{1}{1 + e^{-(y+iy-k)}}(1 - ay) \tag{21}$$

$$b_i = \frac{1}{1 + e^{-(iy-k)}} \tag{22}$$

These are used to generate a measure of the gain from switching given i cooperators:

$$d(i) = a_i - b_i \tag{23}$$

Which gives a gain sequence:

$$\boldsymbol{d} = (d_0, d_1, ..., d_n) \tag{24}$$

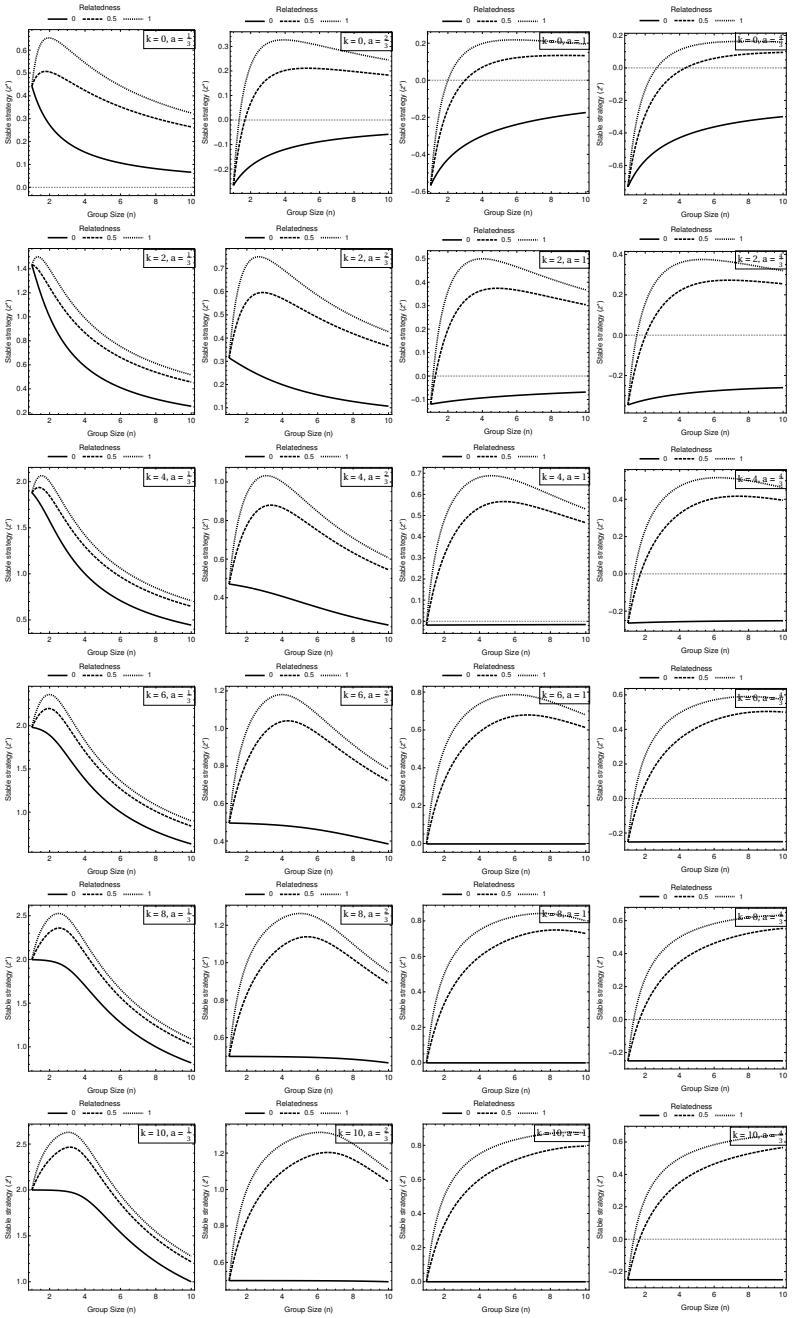
Now the purpose of this process is that the signs of the elements in the gain sequence, $\boldsymbol{d}$, tell us the stability of the two trivial rest states of the system and the number and stability of any interior rest points; assuming evolution occurs in an infinitely large well-mixed population (Peña et al., 2014). We are interested in three properties of the sequence:

1. If the sign of the first element (d_0) is negative then the rest state of full defection is stable.

2. If the last element of the sequence (d_n) is positive then the rest point of full cooperation is stable.

3. If there is one sign change in the sequence then there exists a unique interior rest point, furthermore, if the first element is positive then both trivial rest cases (all defect, all cooperate) are unstable and the interior point is stable.

Figure 6 shows the case with the parameters: $a = \frac{2}{3}$, $n = 10$, and $k = 2$. There exists a parameter range for the cooperating strategy between $z = (0.1, 0.5]$ where there is a stable interior rest point — cooperators and defectors co-exist. When the trait is sufficently low then there is stable point when the population is all cooperators and when the trait value is higher than 0.5 the only stable scenario is all defectors. From the equilibrium game before we might expect the toxin production value to be around $z* = 0.107$ (3s.f.). This gives an initial element to the gain vector of, $d_0 = 0.00237$ (3s.f.), and a final element of, $d_0 = 0.000457$ (3s.f.), with no sign change in between. This indicates that the ESS solution to the static game would give a fully cooperative equilibrium in the dynamic game given these parameters. Further, from Peña and Nöldeke (2018) (proposition 1) we can see that as group sizes increase the equilibrium proportion of cooperation will decrease.


Relatedness
0
0.5
1
k = 0, a = 1/3
k = 0, a = 2/3
k = 0, a = 1
k = 0, a = 4/3
k = 2, a = 1/3
k = 2, a = 2/3
k = 2, a = 1
k = 2, a = 4/3
k = 4, a = 1/3
k = 4, a = 2/3
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k = 6, a = 1
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k = 8, a = 1/3
k = 8, a = 2/3
k = 8, a = 1
k = 8, a = 4/3
k = 10, a = 1/3
k = 10, a = 2/3
k = 10, a = 1
k = 10, a = 4/3
Stable strategy (x*)
Group Size (n)

Figure 5

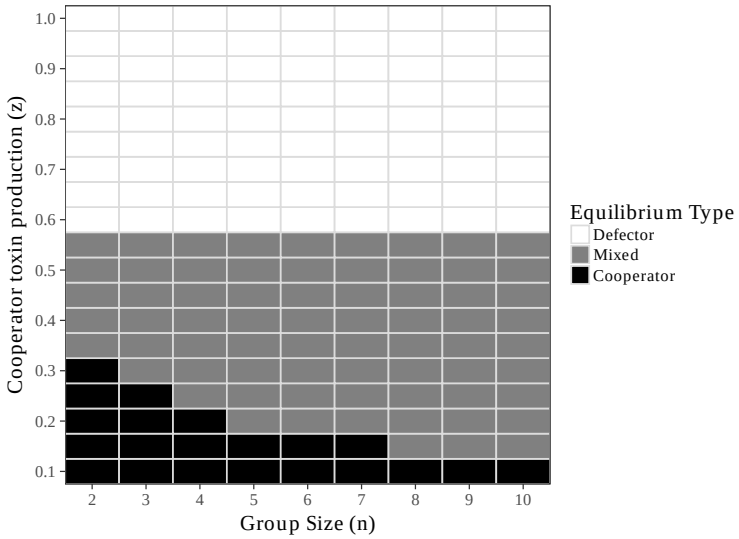


Figure 6: This figure shows the dynamics of a population of cooperators and defectors as described by eq. (10). Each point represents a poulation with n group size and cooperators that produce z toxin. Using the criteria for the gain sequence for each population we cklassify it as either a defector only equilibrium a cooperator only one or a mixed equilibrium where the two strategies coexist. The graph was drawn using the parameters, $a = \frac{2}{3}$ and $k = 2$ (appendix D).

3

DYNAMICS OF BT

Combining social evolution and disease ecology in *Bacillus thuringiensis*

Matishalin Patel, Michael Bonsall, and Stuart West

1 Abstract

Evolutionary and ecological timescales can coincide. This leads to feedbacks where evolutionary changes in gene frequencies directly impact the short term population dynamics and vice-versa. We model a key example of these kinds of feedbacks using the insect pathogen *Bacillus thuringiensis*, which engages in a two strategy public goods game which determines the success of an infection. We model both strategies, as separate genetic strains, and a host population using discrete time equations. We show that the changes in strain frequencies are influenced by ecological factors and influences those factors in turn. We also show that in the long term these dynamics can lead to intermittent fluctuations in strategy frequencies and bacterial population densities and not to stable equilibria.

keywords:

virulence, cooperation, game theory, kin-selection, evolution, social evolution

2 Introduction

Ecological and evolutionary processes are often considered to act on different timescales. However, there has been increasing focus on how changes in ecological processes can have a rapid effect on evolutionary processes (Ellner et al., 2011; Sutherland et al., 2013). Bacteria are able to undergo rapid evolutionary changes and also engage in many social behaviours that can have significant effects on population dynamics (West et al., 2007). The effect of these ecological and evolutionary dynamics interacting at short timescales are thought to lead to unpredictable or unexpected results. Many bacterial population dynamics are affected by public goods games between cells. These games can show density and frequency dependent effects (Ross-Gillespie et al., 2007; Ross-Gillespie et al., 2009; Gore et al., 2009). When these occur this can lead to much more complicated outcomes for population dynamics than if either the social games or the population dynamics were considered in isolation (Sanchez and Gore, 2013; Gokhale and Hauert, 2016).

The bacteria *Bacillus thuringiensis* produces a Crystal toxin to invade and overcome the larval stages of several insect species (Höfte and Whiteley, 1989). *B. thuringiensis* is used as a biocontrol for pest species such as the Diamondback moth (*Plutella xylostella*). The production of the toxin is a volunteer's dilemma for the bacteria, a group of bacteria once ingested need to produce a sufficient amount to perforate the gut of the host but once perforated any bacteria can take advantage of the hole (Archetti, 2009; Raymond et al., 2012; Raymond and Bonsall, 2013; Patel et al., 2019). The social dynamics surrounding this game therefore impact the persistence and virulence of a population of bacteria sprayed onto a field or resident in the soil of a farm (Raymond et al., 2012; Raymond and Bonsall, 2013).

The Crystal toxins are metabolically expensive therefore there is selection for individuals to become non-producers (defectors in the game) (Loferer-

Krößbacher et al., 1998). The dynamics of these two strategies (cooperators and defectors) can be understood using social evolution theory (Archetti, 2009; Patel et al., 2019). Simple analytical models predict a stable level of cooperation should exist, there is some unbeatable cooperative strategy. However, experimental data has shown that the amount of toxin produced by isolates of *B. thuringiensis* is highly variable, both over a growing season and between sites in the same locality (Raymond et al., 2010). This suggests that evolution is driving the development of different levels of cooperation in response to both environmental and evolutionary factors.

The overall patterns will depend on how evolution in response to the game affects the population dynamics of both the bacterium and the insects it infects, if defectors are common in the bacterial population virulence is reduced. In social games such as the volunteer's dilemma density and frequency can affect the evolution of defection (Patel et al., 2019). Firstly, when populations are dense, defection (not-producing the toxin) becomes favoured because there is a high chance that defectors will still receive a benefit from all the neighbouring cooperators, equally when density is low, cooperators (producers) are favoured because they are more self sufficient (Ross-Gillespie et al., 2009). Secondly, when cooperators are most frequent, defectors are likely to interact with cooperators rather than other defectors so they are favoured by natural selection, equally when defectors are the most frequent, they are disfavoured because they will interact mostly with other defectors (Ross-Gillespie et al., 2007). This might lead to low bacterial densities in the future which means we might expect cooperators to reinvade. This then increases density and decreases defector frequency which suggests defectors should now invade the population and cause the cycle to repeat.

We build a set of simple models to explore how this feedback between social evolution of the trait and the disease ecology interact. By linking the

social evolution of the bacteria with a simple model of its population dynamics we try to determine what patterns of cooperation and defection we should observe and which important factors, such as density and frequency, drive them.

3 Models

We consider three classes of individuals in our models cooperating bacteria (C) that produce an amount of toxin $z_c \in [0,1]$, defecting bacteria (D) which produce an amount of toxin $z_d = 0$, and susceptible insect hosts (S). Our first model keeps these susceptible hosts constant and only considers the dynamics of the cooperators and defectors. The second model includes all three classes. We use the second model to then look at the dynamics between years by mapping the population sizes of cooperators and defectors at the end of the year to the initial conditions at the start of the next year.

3.1 Cooperator-Defector Model

In the bacteria only model we model two strains of bacteria, a number of cooperators, C, and a number of full defectors, D. We assume a constant population size of hosts, S. These bacteria infect the hosts in groups of size P. We model the distribution of group sizes using a poisson distribution where the mean (rate parameter) is the per host number of bacteria:

$$P \sim \text{Poi}\left(\frac{C+D}{S}\right). \tag{1}$$

Within each group we assume that groups are non-random. We use two composed Poisson Binomial distributions (Bimodal Poisson Binomial distribution). A Poisson Binomial is a modified binomial distribution where each draw has a different probability. In the first draw we use simply the probability of being either a cooperator or a defector; all future draws are

biased by some small amount (ϕ) in favour of whatever type was initially drawn. Therefore, if the first member of a group is a cooperator than all other members have a higher chance of also being cooperators. This mimics a spatial structuring where groups aggregate. The distribution of number of cooperators (n) within these groups is then (appendix A.1):

$$N_C \sim \text{BiPoiBin}\left(P, \frac{C}{C+D}, \phi\right) \tag{2}$$

So the expected number of cooperators in a group is then:

$$\sum_{m=1}^{\infty} \mathbb{P}(P = m) \sum_{i=1}^{m} \mathbb{P}(N = i \,|\, P = m) \tag{3}$$

If we know the number of cooperators in a group we can calculate the chance that group successfully invades the host $\beta(.)$. We adapt the functions from Patel et al. (2019):

$$\beta(z, i) = \frac{1}{1 + e^{iz-h}}. \tag{4}$$

Where, z is the amount of toxin each cooperator produces, i is the number of cooperators, and h is the threshold amount necessary. We also assume a simple linear cost to the production of toxin:

$$f(z) = 1 - az, \tag{5}$$

where, a is the cost per unit toxin.

Combining these equations we can write out a difference equation to map the number of cooperators, C, and defectors, D, from one timestep to the next. First, we calculate how many new bacteria are added by infection,

given by the expectation:

$$C_t^{\text{inf}} = \sum_{m=1}^{\infty} \mathbb{P}(P = m) \sum_{i=1}^{m} \mathbb{P}(N_C = i)\beta(z, i)f(z)\lambda_C Z_t S_t, \tag{6}$$

where,

$$P \sim \text{Poi}\left(\frac{C_t + D_t}{S}\right),$$

$$N_C \sim \text{BiPoiBin}\left(m, \frac{C_t}{C_t + D_t}, \phi\right).$$

The term λ is the burst size. Z is the collision rate which is determined by using the number of bacterial groups (g) and the number of susceptible hosts (S) their relative densities are multiplied to give a collision rate:

$$g_t = \frac{C_t + D_t}{S_t}$$

$$Z_t = \frac{g_t}{S_t + g_t} \frac{S_t}{S_t + g_t}. \tag{7}$$

By extension we have the equivalent expression for the distribution of defectors (D):

$$D_t^{\text{inf}} = \sum_{m=1}^{\infty} \mathbb{P}(P = m) \sum_{i=1}^{m} \mathbb{P}(N_D = i)\beta(z, m - i)f(0)\lambda_D Z_t S_t, \tag{8}$$

where,

$$P \sim \text{Poi}\left(\frac{C_t + D_t}{S}\right),$$

$$N_D \sim \text{BiPoiBin}\left(m, \frac{D_t}{C_t + D_t}, \phi\right).$$

Equations (7) and (9) give the expected number of newly created spores for each strain. We also calculated the expected number of bacteria that became committed to a host (ingested by it, C^{com} and D^{com}). These spores are removed from the starting population as it is assumed that if they fail

to invade the host that they die:

$$C_t^{\text{com}} = \sum_{m=1}^{\infty} \mathbb{P}(P = m) \sum_{i=1}^{m} \mathbb{P}(N_C = i) S_t Z_t i \,. \tag{9}$$

Therefore we can express the total change in the population of cooperators and defectors as:

$$C_{t+1} = \Big((C_t - C_t^{\text{com}}) + C_t^{\text{inf}} \Big)(1 - \mu) \tag{10}$$

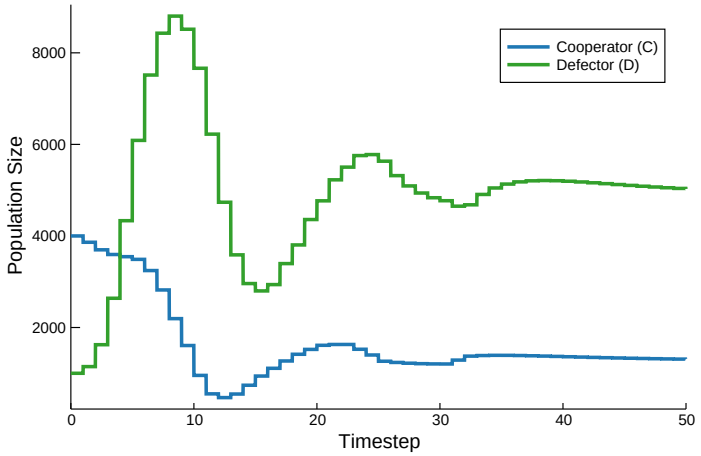
$$D_{t+1} = \Big((D_t - D_t^{\text{com}}) + D_t^{\text{inf}} \Big)(1 - \mu). \tag{11}$$

Where, μ is the per capita death rate of the bacteria. These difference equations were used to determine the dynamics when we assume host populations are not dynamic. Instead we keep the host population constant at some carrying capacity ($S_t = K$).

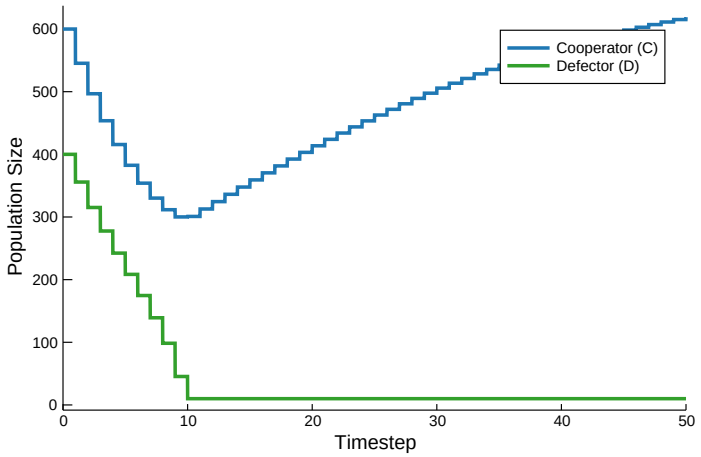
Figure 1 shows some example dynamics of our model. Depending on the parameters the model shows damped oscillations, stable co-existence or exclusion. We solved for the end state of the cooperator and defector population sizes after four times steps. Four time steps means there was four generations of insects each year which the bacteria infect. This gives an idea of the final population state after a single year.

We focused on three key parameters in the model: (i) the starting bacterial density (d), we expect high densities to favour defectors; (ii) the initial frequency of defectors (f), we expect defectors to spread at low frequencies (negative frequency dependence); (iii) the level of aggregation (ϕ), the more individuals aggregate the less easy it is for a defector to take advantage of a cooperator. As the response variables we looked at the proportion of defectors at the end of the year, this indicates the evolutionary trajectory the population is taking. We also looked at the density of bacteria to hosts which gives us an idea of the population dynamics occurring.

For the density of bacteria we limited the exploration to between 1 and



(a)



(b)

Figure 1: **(a)** With an initial density of $d = 5$ and initial defector frequency of $f = 0.2$, both of which favour defection at the beginning. We see damped oscillations towards an equilibrium between cooperators (blue) and defectors (green). **(b)** Dropping the initial density to $d = 1$, which disfavours defectors and increasing defector initial frequency to $f = 0,4$, also disfavouring defectors, we see that defectors are driven to extinction.

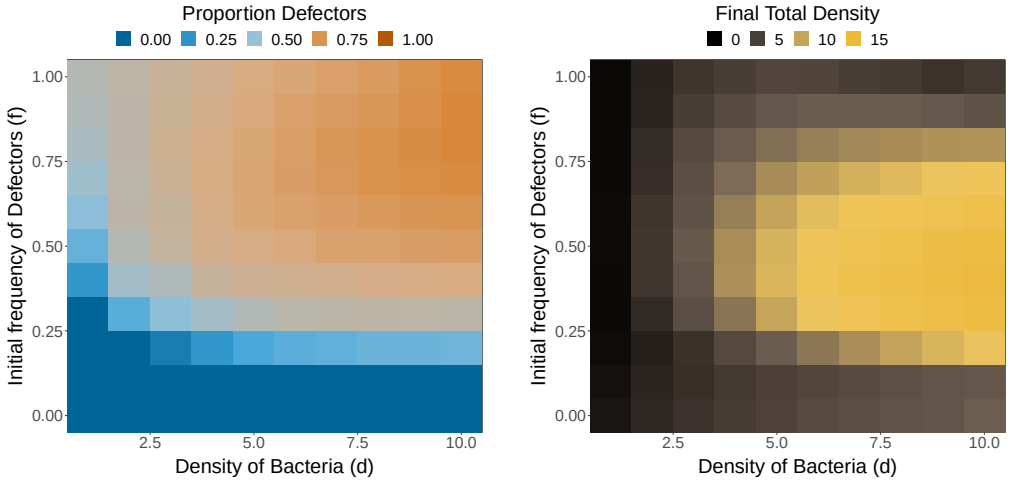
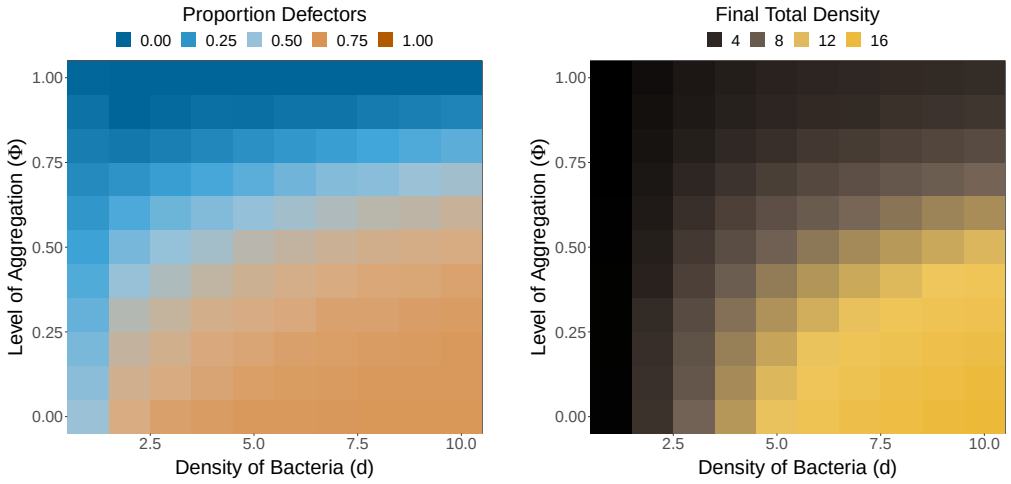


Figure 2: **(a)** The initial density of bacteria and the initial frequency of defectors interacts to give the final proportion of defectors after one year (4 timesteps). At low densities defectors cannot invade well even when at high initial frequencies. As density increases defector frequency increases with increasing initial frequency. **(b)** When looking at total population sizes we see that at low initial densities overall bacteria populations are small. At higher initial densities they increase in size, however at high and low initial frequencies of bacteria the populations are smallest. At high defector frequencies this appears to be due to low virulence at low defector frequencies defectors fail to invade. At the intermediate values defectors invade and cause a spike in bacterial population size. Aggregation was held at 0.3 for both plots.

10. This is because at around densities of 10 each host is infected by on average 10 spores which is comparable to the most one would expect in nature (Raymond et al., 2010). For frequency, f, and aggregation, ϕ, we considered the range $[0,1]$ at regular intervals (table 1).

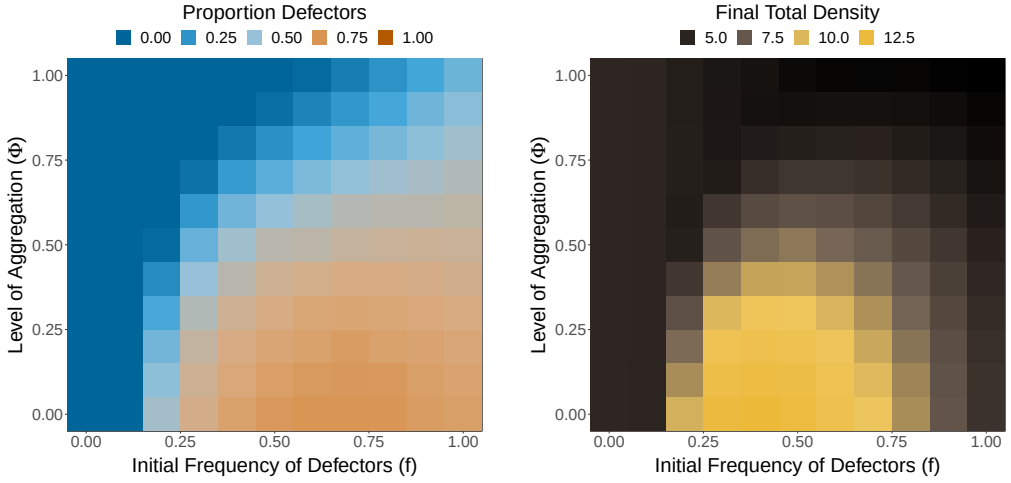
Figure 2 shows how the frequency and density of bacteria interact. Higher initial densities lead to a higher final proportion of defectors. Higher initial frequencies of defectors also lead to higher defector frequencies at the end of the year. Density also increased population sizes but in a region bounded by the frequency of defectors. Intermediate frequencies of defectors invade the population and cause a spike in bacterial densities by out-competing the cooperators.

Aggregation has a strong effect on the frequency of defectors and the density of bacteria. Low aggregation combined with high densities increased defector frequency and population densities the most (fig. 3). Frequency of



(a) (b)

Figure 3: **(a)** High initial densities of bacteria and low levels of aggregation favour defectors as they are likely to be assorted into cooperator dense groups. **(b)** Final population densities were also largest when initial density was high and aggregation was low. Initial frequency was held at $f = 0.5$ for both plots



(a) (b)

Figure 4: **(a)** High levels of aggregation suppressed the frequency of defectors. However this effect was less powerful when initial defector frequencies started high, probably due to the short evolutionary timescale. **(b)** Final population densities were largest when aggregation was low and initial defector frequency was intermediate. This allowed defectors to invade and cause a spike in bacterial density. Initial density was held at $d = 7$ for both plots

defectors interacted slightly differently with aggregation (fig. 4). High levels of aggregation still limited both defector frequency and bacterial abundance, however high defector initial frequency overcome the effects of aggregation somewhat allowing for high defector frequencies and bacterial abundances over a larger range at higher defector initial frequencies.

3.2 Cooperator-Defector-Susceptible Model

In the previous model the number of hosts (S) was held constant. We now specify an equation to govern the growth of hosts in the environment. A simple model for population growth is Ricker's growth equation:

$$S_{n+1} = S_t \exp\left[r\left(1 - \frac{S_t}{K}\right)\right]. \tag{12}$$

Where, r is the base growth rate, and K is the carrying capacity of the population. However, we would like to include the impact of bacterial infection by only considering uninfected hosts ($\overline{S}_t$):

$$\overline{S}_n = \sum_{m=1}^{\infty} \mathbb{P}(P = m) \sum_{i=1}^{m} \mathbb{P}(N = i)(1 - \beta(z, i))Z_t S_t, \tag{13}$$

where,

$$P \sim \text{Poi}\left(\frac{C_t + D_t}{S}\right),$$

$$N \sim \text{BiPoiBin}\left(m, \frac{C_t}{C_t + D_t}, \phi\right).$$

So the growth of the hosts becomes:

$$S_{t+1} = \overline{S}_t \exp\left[r\left(1 - \frac{\overline{S}_t}{K}\right)\right]. \tag{14}$$

Using this growth equation we can now simulate the interaction of all three parties. Figure 5 shows some of the example dynamics with all three populations modelled. There are epidemic spikes in bacterial population

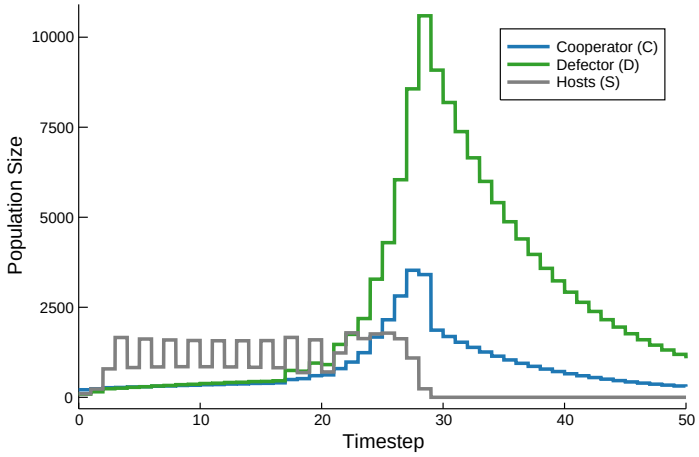
sizes that cause the host population to crash. We also see some co-existence of the host and the bacteria. We considered growth rates of 2.5 and 3 these are large and put the host dynamics into a oscillatory regime, however, because of the high mortality in our model lower values would lead to regular extinctions.

For the interaction of starting densities and initial frequencies of defectors we saw similar results to the Cooperator-Defector model for the final defector frequencies. Defectors were favoured at high densities and intermediate initial frequencies (fig. 6). However, there were differences in final population sizes. Population size in the Cooperator-Defector-Susceptible model was largest at intermediate densities rather than at maximum densities. This is due to the suppression of the host population growth when bacteria are at high densities.

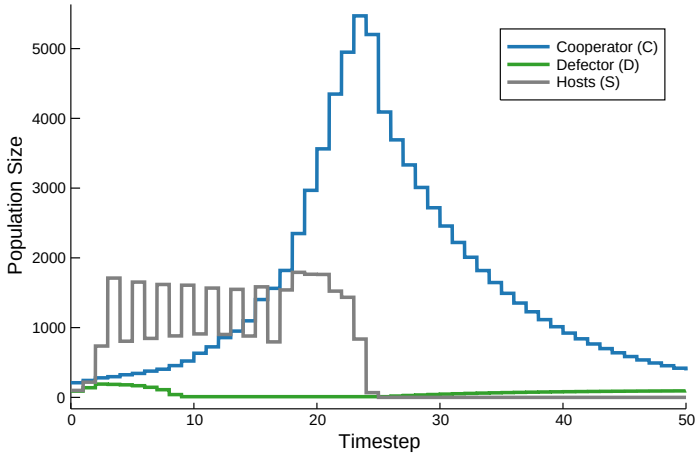
Aggregation overall had a much stronger effect in the CDS model. High levels of aggregation lowered both bacterial abundance and defector frequency (fig. 7). There was an interaction between aggregation and frequency wherein, at intermediate initial frequencies, bacterial abundance was maximal with a small but non-zero level of aggregation (fig. 8). At low aggregation and intermediate defector frequencies defectors sometimes gain more than they lose from the increase in group quality due to cooperator aggregation (Patel et al., 2019).

3.3 Year on Year

We predict that these effects of of the within year dynamics of population density and frequency should lead to fluctuations in the density of bacteria and the proportion of defectors. To demonstrate these effects we iterate over years using the Cooperator-Defector-Susceptible model to get the end of year numbers for defectors and cooperators and use them as the initial conditions for the next year. Specifically, given a final number of coopera-

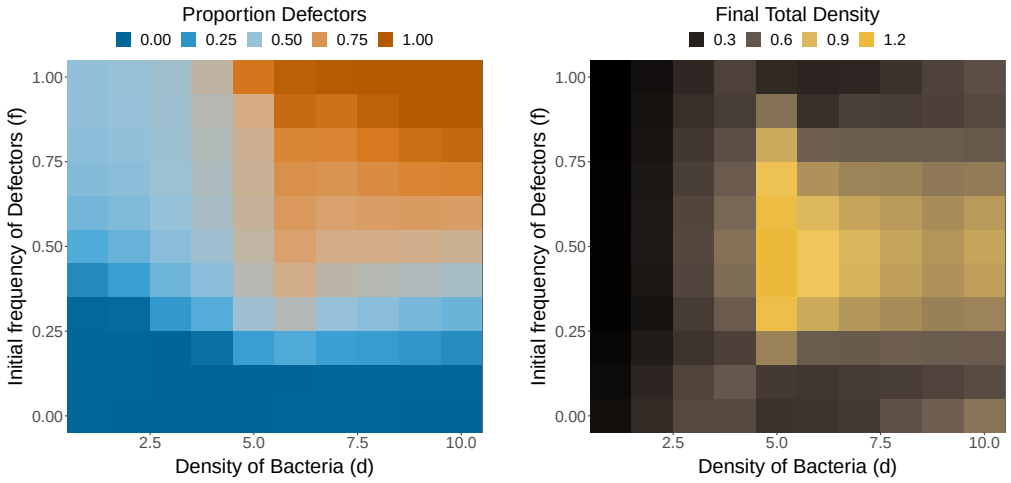


(a)



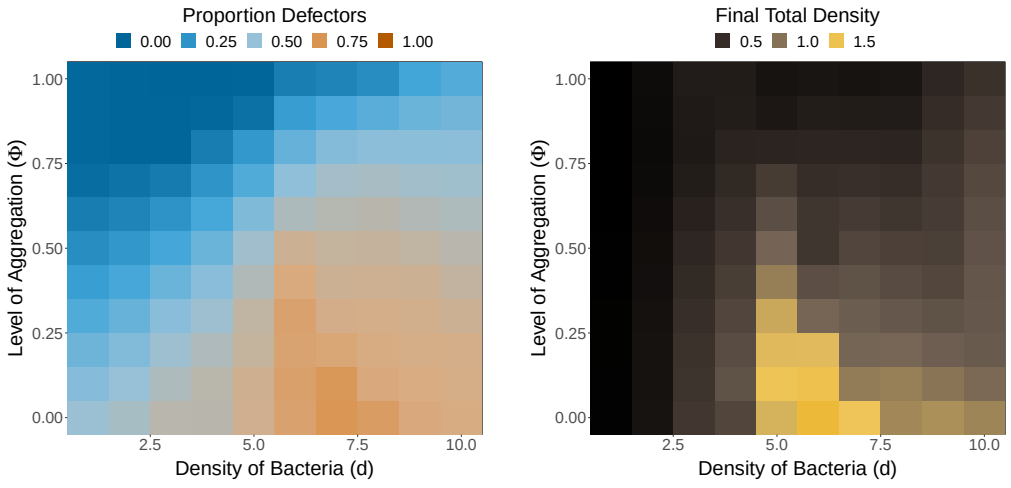
(b)

Figure 5: **(a)** In this example with an initial density of $d = 3$ and an initial frequency of defectors of $f = 0.3$ we see a large epidemic crash the host population after 20 timesteps. The defectors dominate the bacterial population and are at a high frequency afterwards. **(b)** We have now included a small effect of aggregation so that groups are more highly related. The epidemic spike is now dominated by cooperators and occurs more quickly without the defector load. Also host populations are smaller under when cooperators can aggregate and thus infect hosts more efficiently.



(a) (b)

Figure 6: **(a)** The proportion of the defectors at the end of the year is much lower at low initial densities in the Cooperator-Defector-Susceptible (CDS) model. Once again at high densities with high initial frequencies the proportion is highest. **(b)** Final population size is largest at intermediate initial densities and initial frequencies. This differs from the model with only Cooperators and Defectors where increasing initial density always increased population. Aggregation was held at 0.3 for both plots.



(a) (b)

Figure 7: **(a)** High initial densities of bacteria and low levels of aggregation favour defectors in the Cooperator-Defector-Susceptible model. Once again intermediate initial densities seem to maximise the success of the defectors. **(b)** There is a strong effect of low aggregation and intermediate initial densities on the final number of bacteria. However in general aggregation seems to suppress bacterial density strongly except in this small area, as the populations are either mostly cooperators or collapse due to high defector frequency. Initial frequency was held at $f = 0.5$ for both plots

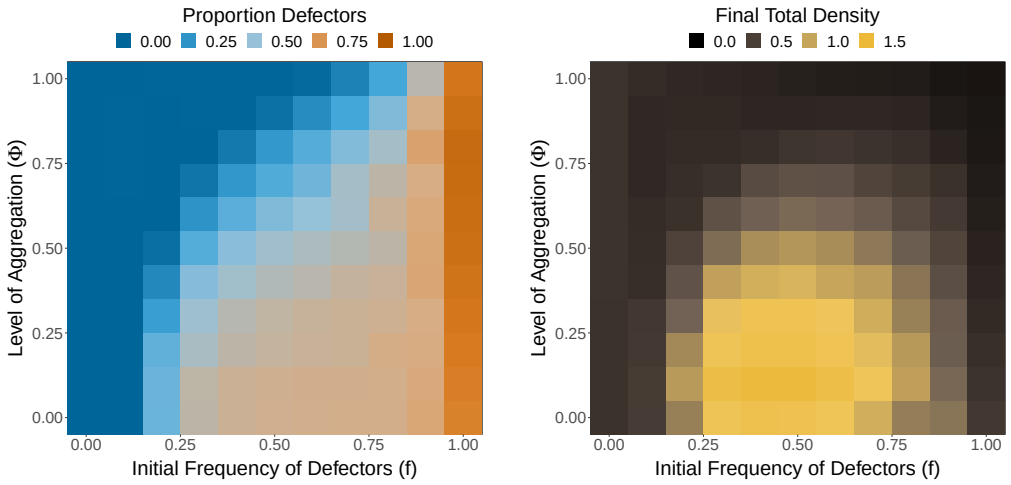


Figure 8: **(a)** Increasing the initial frequencies of defectors allowed them to persist at higher levels of aggregation. In general aggregation suppressed defectors strongly though. **(b)** For straight population size the maximum size was actually reached at intermediate initial frequencies and a low but non-zero level of aggregation. This is due to that level of aggregation not fully excluding the defectors from groups and letting them hitchhike efficiently on cooperators driving a spike in defector numbers. Initial density was held at $d = 7$ for both plots

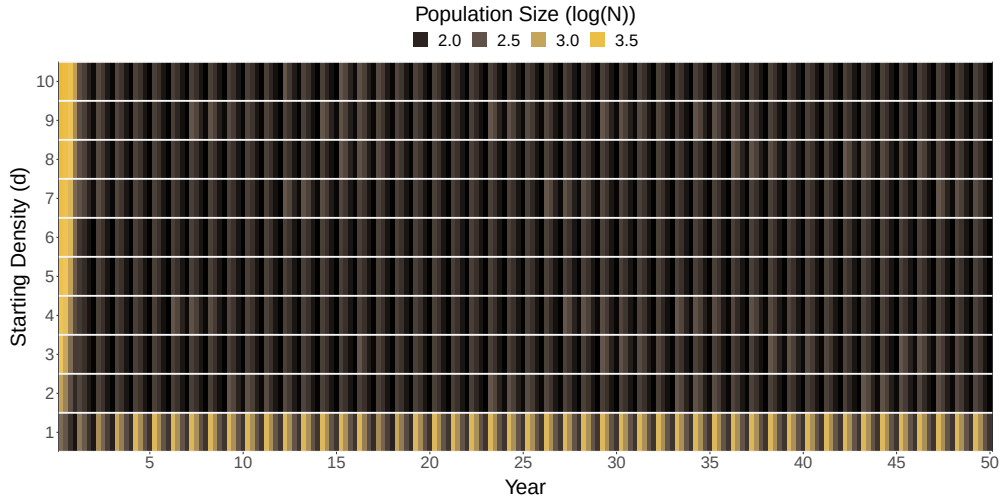
tors (C_f), defectors (D_f), and susceptibles (S_f), the initial density of bacteria (B) and frequency of defectors (F) are:

$$B = \frac{C_f + D_f}{S_f}$$

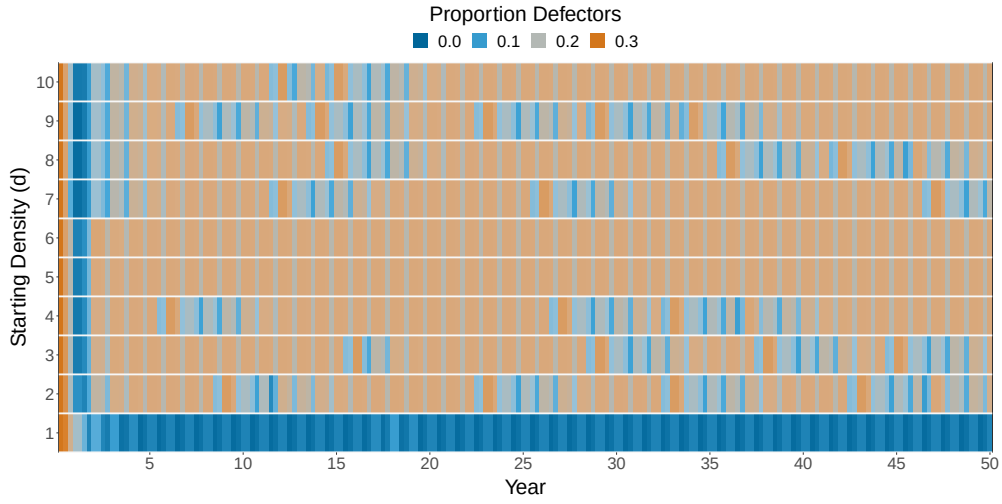
$$F = \frac{D_f}{C_f + D_f}$$

We also assumed that the starting host population was always half of the carrying capacity. This is because we expected between year dynamics to be governed by some larger scale system and the iterated model here is focusing on one patch that will be recolonised a the start of each year by neighbouring host patches.

We iterated the dynamics for 50 years, yielding 200 timesteps, to give an idea of the long term impacts of the initial conditions. We looked at three major impactors of the bacterial dynamics: starting frequency of defectors (f), starting bacterial density (d), and aggregation (ϕ).

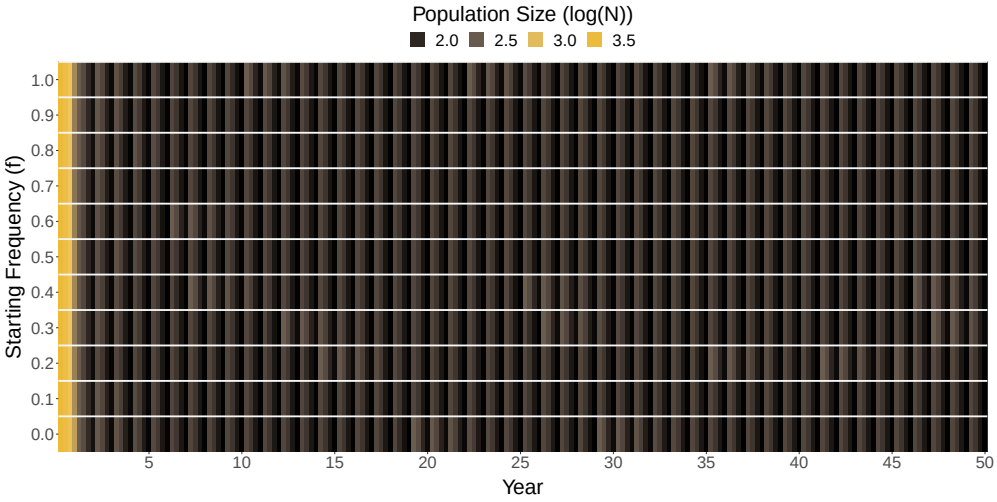


(a)

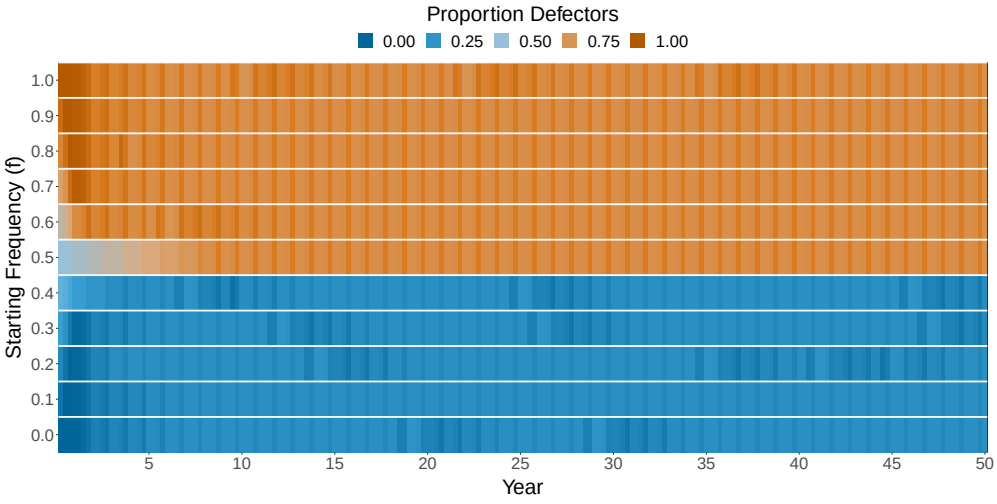


(b)

Figure 9: With initial $\phi = 0.1$, $f = 0.3$. **(a)** Here we can see the effect of starting density on the logged bacterial population size. In general high densities leads to a large initial population size but over time the equilibrium reached is very similar year to year. The exception is when the starting density $d = 1$ occurs then the long term population dynamics range over a wider range of values. **(b)** The proportion of defectors varies unevenly with starting density. There are intermittent periods where defectors become uncommon and cooperators dominate the population. These periods also coincide with slightly higher bacterial densities (lighter blue) in (a).

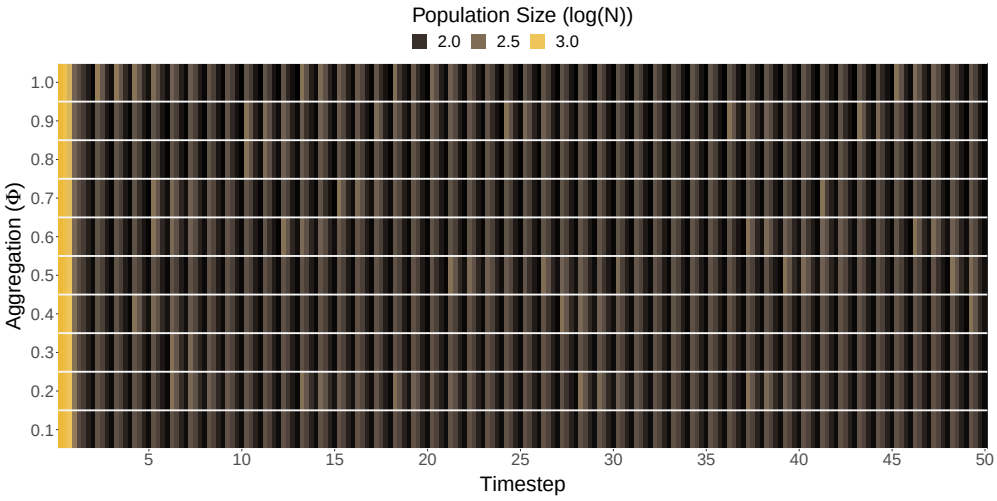


(a)

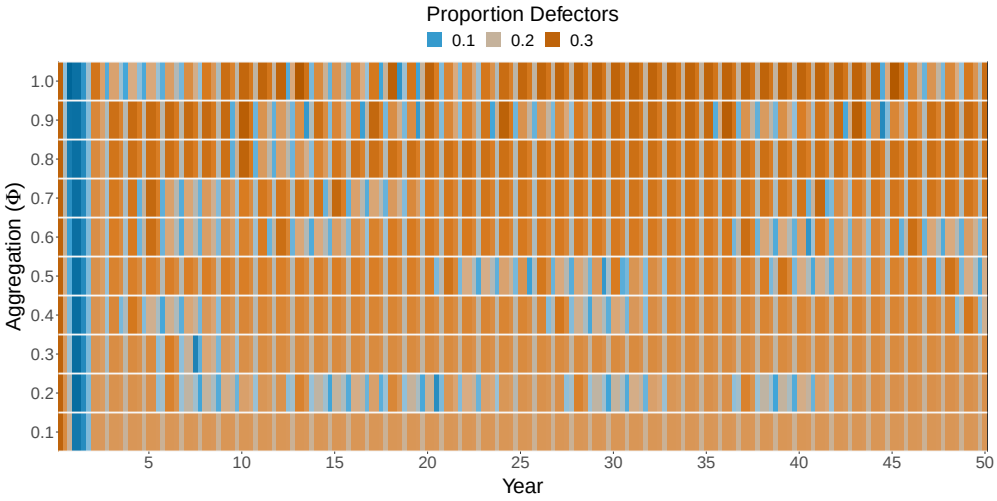


(b)

Figure 10: With initial $\phi = 0.1$, $d = 7$. **(a)** Here we can see the effect of starting defector frequency on the logged bacterial population size. Regardless of starting frequency there is an large initial spike in population size followed by seasonally driven spikes. There is some indication at high and low starting defector frequency that there are intermittent periods of higher population size (lighter segments). **(b)** There is a divide between where starting defector frequency led to high or low defector frequencies. One can again see the spikes in defector or cooperator frequency intermittently in low and high starting frequency patches (darker segments within a year).



(a)



(b)

Figure 11: With initial $f = 0.3$, $d = 5$. **(a)** Here we can see the effect of aggregation on the logged bacterial population size. Aggregation did not affect the long term dynamics of the population sizes. High aggregation did seem to lead to more cooperator invasions which did lead to short term changes in population size (lighter segments). **(b)** Aggregation had a similar effect to initial frequency on defector frequency. High levels of aggregation lead to repeated re-invasions (blue segments) of cooperators into the defector dominated populations.

Starting frequency of bacteria had the simplest impact on the long term dynamics (fig. 10). Depending on the starting density and the level of aggregation there was a critical starting frequency above which the long term stable state was dominated by defectors and below which the population was dominated by cooperators.

In fig. 9 the intermediate starting densities led to a constant stable state, which we might expect as that is also where defectors will easily dominate the population. However, at low or high starting densities the interactions between cooperators and defectors were not stable leading to intermittent sections where over the course of 3-5 years cooperators would invade the population and then be selected back out.

For aggregation (fig. 11) the pattern was very similar to that for density. In the short term aggregation lead to a strong dominance of cooperators in the first two years. However in the long term these cooperator heavy states devolved into intermittent cycling between cooperator and defector dominated populations. Whenever cooperators invaded the population they also drove up population density which allowed defectors to re-invade.

4 Discussion

We analysed a two party Cooperator-Defector model and a more complicated three party Cooperator-Defector-Susceptible model. We also looked at the dynamics as the results from the endpoint of the Cooperator-Defector-Susceptible model were fed back into the initial conditions for the next year.

In the simple Cooperator-Defector model we found results that closely matched those in previous theoretical and experimental work (Ross-Gillespie et al., 2007; Ross-Gillespie et al., 2009; Patel et al., 2019). Low defector frequency favoured defectors to be increasing in frequency over the year. When bacterial densities were high then total population densities and defector frequency were higher at the end of the year. When aggregation (re-

latedness) was low then defectors were favoured as well as they could interact with more cooperators. These effects could help explain why local variation is so high in *B. thuringiensis* both in time and space (Raymond et al., 2010, 2012; Raymond and Bonsall, 2013).

In the more complicated Cooperator-Defector-Susceptible model we also find the same general patterns with some key differences. The addition of changing host population added a non-monotonic effect to the effects of density and aggregation. Intermediate initial densities led to the highest final densities over low or high initial densities. Also we saw an effect of small, but non-zero, aggregation leading to the highest final population sizes. Both these effects seem to occur because when defectors invade they increase final populations sizes.

In the year-on-year dynamics we see the effect of these simple within year patterns. The long term dynamics were heavily influenced by the initial conditions. Interactions between cooperators and defectors mean that initial conditions do matter for the final equilibrium state. Most strikingly the dynamics show that over a wide range of densities and levels of aggregation the frequency of defectors can fluctuate intermittently. Cooperators invade the population, driving down the defector frequency. This however leads to re-invasion as defectors become less common and density increases. These cycles of invasion and re-invasion occur over the span of multiple years.

When viewed over the 50 years we ran the simulations there does not seem to be any strong pattern in the periodic occurrence of these invasions. This suggest the dynamics could be chaotic. Using methods such as Fourier analysis to create a power spectrum or more fine-grained explorations of parameter space might give a clearer picture of whether these patterns are chaotic or deterministic and if there are any strong correlations between parameter values and the regularity of these invasions.

The results from these models suggests that within year evolutionary and ecological dynamics can drive longer term patterns. The high variance in strategies of field samples of *Bacillus thuringiensis* and the varying spore counts could be the result of the interaction between the social evolution of these bacteria and the ecological dynamics of their epidemiology. Also, these patterns may be chaotic in nature as they are sensitive to initial conditions and amplify small differences year-to-year. Predicting short term changes may be possible, but we may be unable to give accurate predictions on future states even with near perfect information about the bacterial and host populations.

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A Appendix

A.1 Bimodal Poisson Binomial

We use a modified Poisson binomial distribution to model the aggregation of groups. The Poisson Binomial models a number of draws where the success of each draw is different. The Poisson Binomial distribution takes in a vector of length k for a distribution over k draws and each element in k is the probability for success on that draw, the first element being the first draw and so on.

The Poisson Binomial then for the number of cooperators in a group of m individuals is then:

$$\text{PoiBin}([c_1, c_2, \ldots, c_m]) \tag{A.1}$$

We assume that the all draws after the first draw are biased towards cooperators given the first draw is a cooperator. If all groups are started at random and there is c probability that an individual is a cooperator then:

$$c\ \text{PoiBin}([1,\ c_2 + \delta_1, \ldots, c_m + \delta_1]). \tag{A.2}$$

Where, δ_1 indicates the added bias towards cooperators. equally for defec-

tors (d):

$$d \text{ PoiBin}([0,\ 1-(d_2+\delta_2),\ldots,1-(d_m+\delta_2)]). \tag{A.3}$$

Where, δ_2 indicates the bias towards defectors. The sum of these two distributions gives the overall distribution for cooperators in the population. We calculate δ_1 and δ_2 as:

$$\delta_1 = \phi * (1-p) \tag{A.4}$$

$$\delta_2 = \delta_1 * p \tag{A.5}$$

The distribution of cooperators in the populations is then:

$$\begin{aligned}\text{BiPoiBin}(m,c,\phi) = & c \text{ PoiBin}([1,\ c_2+\delta_1,\ldots,c_m+\delta_1]) + \\ & d \text{ PoiBin}([0,\ 1-(1-c_2+\delta_2),\ldots,1-(1-c_m+\delta_2)])\end{aligned} \tag{A.6}$$

This allows strongly Bimodal distributions of groups. Where the majority will be in groups with the same type as them while maintaining the same mean group composition as a normal binomial.

B Simulations

The simulations were run in Julia 1.1 using the JuliaDiffEq collection of packages. They were run locally on a Dell OptiPlex 7040 in an Ubuntu 18.04 environment. Data was processed in R 3.6.1 using the tidyverse package.

For the simulation we considered a restricted range of parameters due to the high dimensionality of the system. In the plots the default parameters were: $d = 5$, $f = 0.5$, $\phi = 0.3$, $z_c = 0.5$, $a = 0.9$.

Parameters
$d \in \{1, 2, \ldots, 10\}$
$f \in \{0, 0.1, \ldots, 1\}$
$\phi \in \{0, 0.1, \ldots, 1\}$
$z_c \in \{0.1, 0.5, 0.9\}$
$a \in \{0.1, 0.5, 0.9\}$
$r \in \{2.5, 3\}$
$\lambda_C = 20$
$\lambda_D = 40$
$\mu = 0.1$
$h = 1$
$K = 5000$

Table 1: The parameter ranges for which simulations were carried out.

4

INDISCRIMINATE SPITE

Can natural selection favour indiscriminate spite?

Matishalin Patel, Stuart West, and Jay Biernaskie

1 Abstract

Spiteful behaviours occur when an actor harms its own fitness to inflict harm on the fitness of the recipient. Hamilton (1970) found that in order for spiteful genes to spread the spite had to be directed at individuals who were related to the actor with a negative relatedness. A number of papers have suggested scenarios where indiscriminate spite could be favoured, especially in small populations or small groups. However, it is not clear that a negative relatedness could arise without the harming behaviour being preferentially directed towards less related individuals (kin discrimination). We show that: (1) the evolution of spite requires kin discrimination; (2) previous models suggesting indiscriminate spite involve scenarios where the actor gains a direct, feedback benefit from harming others, and so the harming is selfish rather than spiteful; (3) selfish harming can be favoured most in small populations or groups because this is where the feedback benefit of harming is greatest.

keywords:

spite, inclusive fitness, cooperation, kin-selection, evolution, social evolution

2 Introduction

Spite is the hardest type of social trait to explain. Spiteful behaviour reduces the lifetime number of surviving offspring (fitness) of both the recipient and the performer (actor) of that behaviour (Hamilton, 1970). In terms of Hamilton's rule, $-C + RB > 0$, spite represents the case where there is a fitness cost to the actor (positive C), and a fitness cost to the harmed recipient (negative B), which can only be favoured if the genetic relatedness term, R, is negative (Hamilton, 1970). Understanding the meaning of negative relatedness is therefore crucial for explaining how and why spite evolves.

It has been argued that the evolution of spite requires kin discrimination, allowing the actor to harm individuals in the social group with whom they share relatively low genetic similarity (Foster et al., 2000, 2001; Gardner and West, 2004*a*,*b*; Gardner et al., 2004; Lehmann et al., 2006; West and Gardner, 2010). Specifically, spite can be favoured when harming less-related individuals (primary recipients) reduces competition and therefore benefits more-related individuals (secondary recipients). In this case, negative relatedness arises because the actor is less genetically similar to the primary recipients than to the secondary recipients (Lehmann et al., 2006) . In contrast, without kin discrimination, harming behaviours could not be directed at individuals to whom the actor is negatively related, so indiscriminate spite should be impossible.

Previous theoretical studies have suggested the possibility for indiscriminate spite. We define "indiscriminate" as meaning the trait does not affect other individuals differentially based on their kinship. Hamilton (1970) found that non-trivial negative relatedness will arise in any small population, and this led to the prediction that indiscriminate spite could be favoured in sufficiently small populations (Grafen, 1985; Vickery et al., 2003; Smead and Forber, 2012). Specifically, some authors have suggested that individuals could be favoured to hold territories that are larger than

needed for their own interest ("super-territories"), in order to spitefully exclude others from resources Knowlton and Parker (1979); Pleasants and Pleasants (1979); Parker and Knowlton (1980).

Here, we resolve this disagreement over whether indiscriminate spite can occur. Many harming traits will be costly to primary recipients ($B < 0$) but provide a direct fitness benefit to the actor, because they reduce competition. Consequently, they are selfish ($-C > 0$) rather than spiteful ($-C < 0$) (Hamilton, 1970; West and Gardner, 2010). We hypothesise that indiscriminate harming traits like territory size have been misclassified as spiteful when they are actually selfish. We aim to: (1) determine generally whether indiscriminate harming evolves as a spiteful or a selfish trait; (2) examine how different modelling approaches can change the meaning of negative relatedness and lead to misclassification of harming traits; (3) re-analyse the Knowlton and Parker (1979) territory-size model to determine whether it predicts spiteful behaviour.

3 Harming traits

We first modelled natural selection acting on a harming trait, following the approach of Lehmann et al. (2006). The trait has a fitness effect on a focal actor ($-C$) and on two categories of recipients: the harmed primary recipients and the unharmed secondary recipients who benefit from reduced competition (fitness effects B_1 and B_2, respectively). We assume that fitness effects on the actor, primary recipients, and secondary recipients must sum to zero because of competition for finite resources (Rousset and Billiard, 2000):

$$-C + B_1 + B_2 = 0, \tag{1}$$

implying that any decrease in fitness for one category necessarily means an increase in fitness for another. This model could apply to any finite popu-

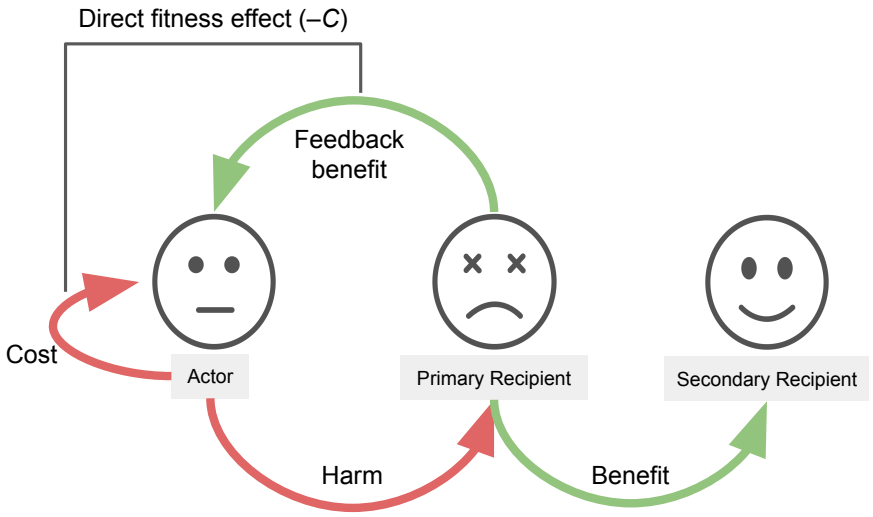

Figure 1: Partitioning the fitness effects of a harming trait. When a focal actor harms a primary recipient, this reduces competition and therefore benefits the unharmed secondary recipients and the actor itself (feedback benefit). Some modelling approaches include the actor in the set of secondary recipients. However, the total direct fitness effect ($-C$ in Hamilton's rule) includes the fecundity cost of expressing the harming trait plus the feedback benefit.

lation of constant size or to a local economic neighbourhood in which there is a zero-sum competition for access to the next generation (Queller, 1994). Examples of such local competition include poly-embryonic wasps competing for resources inside a host, in which a subset of embryos develop into sterile soldiers and kill other unrelated larvae (Gardner and West, 2004*a*; Gardner et al., 2007). In addition, male fig wasps competing for females inside a fig exhibit spiteful harm wherein brothers fight lethally (West and Gardner, 2010).

To predict the direction of natural selection acting on the harming trait, we considered the fate of a mutant harming allele in a population of individuals with a fixed, resident genotype. The success of the mutant allele

depends on its inclusive fitness effect (Hamilton, 1964): the sum of effects from a focal actor's mutant trait on its own fitness and on the total fitness of each recipient category, weighted by their genetic similarity with the actor. Under the usual assumptions of weak selection and additive gene action, the inclusive fitness effect for our model is:

$$\Delta W_{IF} = -C + B_1 Q_1 + B_2 Q_2 \,, \tag{2}$$

where, Q_1 and Q_2 are probabilities of sharing identical genes between the focal actor and a random individual from the primary and secondary recipients, respectively. We note that the fitness effects in eq. (2) could alternatively be weighted by relatedness coefficients, where genetic similarity is measured with respect to a reference population (e.g., $R_i = (Q_i - \bar{Q})/(1 - \bar{Q})$, where $\bar{Q}$ is the average genetic similarity to the whole population, including the actor; Hamilton 1970)

In the following sections, we examine two different ways of defining the category of secondary recipients and therefore partitioning the fitness effects of harming. Both methods correctly predict the direction of selection (they give the same sum as in eq. (2)). The first partitioning also maintains complete separation of direct and indirect (kin-selected) fitness effects ($-C$ and RB, respectively), making it appropriate for classifying harming traits as selfish ($-C > 0$) or spiteful ($-C < 0$). In contrast, the second partitioning obscures the separation of direct and indirect fitness effects, making it inappropriate for classifying traits in this way.

3.1 Is indiscriminate harming spiteful, or selfish?

We determined the conditions for a harming trait to be classified as spiteful or selfish. For this purpose, we assume that the focal actor, primary recipients, and secondary recipients are mutually exclusive categories. This

ensures that the actor is not a recipient of its own behaviour, and so the $-C$ term in the inclusive fitness effect (eq. (2)) captures all effects of the actor's harming behaviour on its own fitness. From eq. (2), we derived the typical two-party version of Hamilton's rule by eliminating the fitness effect on secondary recipients, using $B_2 = C - B_1$ (from eq. (1)). After rearrangement, the inclusive fitness effect is positive, and the harming trait is favoured, when:

$$-C + \frac{Q_1 - Q_2}{1 - Q_2} B_1 > 0\,, \tag{3}$$

which is Hamilton's rule with the relatedness between actor and primary recipients given by $(Q_1 - Q_2)/(1 - Q_2) \equiv R_1$. This is the genetic similarity between the actor and an individual from the potential primary recipients, measured relative to an individual from the potential secondary recipients.

Equation (3) implies that indiscriminate spite cannot evolve. This is because negative relatedness (and hence an indirect fitness benefit of harming) will arise only if harm can be directed at primary recipients who are less similar to the actor than secondary recipients are ($Q_1 < Q_2$). Negative relatedness requires discrimination. However, if the actor were indiscriminate — harming a random subset of a population or local economic neighbourhood — then its expected similarity to these primary recipients would be the same as to the set of potential secondary recipients ($Q_1 = Q_2$), and relatedness would be zero ($R = 0$). This implies that indiscriminate harming will be favoured when it is a selfish trait with a positive direct fitness benefit ($-C > 0$).

3.2 Why does misclassification occur?

Misclassification of harming traits can occur because the fitness effects of social traits can be partitioned in different ways (Frank, 1998). An alternative way of partitioning the effects of harming is to include the actor in the

set of secondary recipients who may benefit from reduced competition. In fact, it is often implicitly assumed that the set of potential secondary recipients is the entire population (or economic neighbourhood), including the focal actor (Hamilton, 1970, 1971; Grafen, 1985; Vickery et al., 2003; Smead and Forber, 2012). To make this explicit, we re-write the inclusive fitness effect as:

$$\Delta W_{IF} = -c + b_1 Q_1 + b_2 \bar{Q}. \tag{4}$$

We use lower-case letters to indicate that the fitness effects no longer match those from eq. (2) but they are still zero-sum. Hence, b_2 is now the benefit of reduced competition that may be experienced by all individuals in population (including the actor), and $\bar{Q}$ is the probability of genetic identity between the focal actor and a random individual in the entire population (including itself). It follows that $-c$ is not a total direct fitness effect because it excludes the secondary benefit of harming that feeds back to the focal actor ().

We used eq. (4) to derive an analogue of Hamilton's rule, which reveals a different version of negative relatedness. For example, in a population (or economic neighbourhood) of N individuals, an actor could indiscriminately harm a random subset of individuals with genetic similarity Q_1 to the actor. If the entire population is in the set of secondary recipients, then the expected genetic similarity between the actor and these recipients is $Q_2^p = \frac{1}{N}1 + \frac{N-1}{N}Q_1$ (where the first term accounts for the actor's similarity to itself). Eliminating the fitness effect on secondary recipients (using $b_2 = c - b_1$), shows that indiscriminate harming is favoured when:

$$-c + \frac{-1}{N-1} b_1 > 0. \tag{5}$$

Where $-1/(N-1)$ is the relatedness between actor and primary recipients,

measured with respect to the entire population, $(Q_1 - \bar{Q})/(1 - \bar{Q}) \equiv R_{1,p}$. This is the version of negative relatedness that has led to predictions of indiscriminate spite in small populations (Hamilton, 1970; Grafen, 1985).

However, although the term $\frac{-1}{N-1}b_1$ resembles an indirect fitness benefit ($RB > 0$), it actually accounts for the secondary benefit of harming that feeds back to the focal actor. Another way of seeing this is to derive an analogue of Hamilton's rule from eq. (4), this time eliminating the fitness effect on primary recipients (using $b_1 = c - b_2$). For example, in a well-mixed population of N individuals, indiscriminate harming is favoured when:

$$-c + \frac{1}{N}b_2 > 0\,, \tag{6}$$

where, $1/N$ is the relatedness between actor and the entire population (including itself), measured with respect to primary recipients ($(\bar{Q} - Q_1)/(1 - Q_1) \equiv R_{2,p}$). The term $(1/N)b_2$ accounts for the fraction of the secondary benefit (reduced competition) that feeds back to the focal actor, which gets larger as the actor makes up a larger fraction of the population.

Our key distinction here is that harming behaviours can be either beneficial or costly to the actor ($-C > 0$ or $-C < 0$), whereas spiteful behaviours are strictly costly to the actor ($-C < 0$). We showed that indiscriminate harming, when it is favoured, is favoured because it is directly beneficial to the actor ($-C > 0$). Moreover, indiscriminate harming will be most favoured in small populations (or small economic neighbourhoods) because this is where the focal actor can benefit most from reducing competition.

3.3 Re-visiting super-territories

We next re-examined the territory size model from Knowlton and Parker (1979) and Parker and Knowlton (1980). We first analysed the model to fully separate direct and indirect fitness effects (applying eq. (2)), asking

whether the model predicts selfish behaviour, as expected. We then used the alternative approach (applying eq. (4)) to illustrate why previous studies have interpreted territory size as a spiteful trait.

We considered a finite, deme-structured population with $d \in \mathbb{Z}^+$ demes and $n \in \mathbb{Z}^+$ individuals competing for territory in each deme; total population size is $N = dn$ (Wright, 1943). Individuals that secure a territory have offspring and then die, afterwards a fraction, m, of their offspring disperse randomly throughout the entire population. All individuals have a genetically-determined strategy for the size of territory that they try to obtain (a continuous trait). Taking over a larger territory has three key effects: (1) it incurs a fecundity cost for the actor (we assume a linear cost with increasing trait size, with slope $a \in [0, 1]$; (2) it harms the actor's deme mates by taking resources away and reducing their fecundity; (3) it reduces the competition to secure a territory in the next generation, faced by non-migrating offspring.

We first assumed that the actor, primary recipients, and secondary recipients are mutually exclusive categories (eq. (2)). In Appendix A, we derive an expression for the fitness, W, of a focal actor as a function of its own territory-size strategy, x; the average strategy of its deme mates (primary recipients), y; and the average strategy of individuals in other demes (secondary recipients), z. We used this neighbour-modulated fitness function to derive the inclusive fitness effect, by taking partial derivatives with respect to the strategies of the different categories of individuals (Taylor and Frank, 1996; Rousset and Billiard, 2000):

$$\Delta W_{IF} = \frac{\partial W}{\partial x} + \frac{\partial W}{\partial y} Q_1 + \frac{\partial W}{\partial z} Q_2 \tag{7}$$

$$= -C + B_1 Q_1 + B_2 Q_2 \tag{8}$$

where, all partial derivatives are evaluated in a monomorphic population

($x = y = z$) with respect to a dummy variable g. In Appendix B, we derive expressions for Q_1 and Q_2, and with these we determined the equilibrium of the model ($\hat{z}$, where directional selection stops) by solving $\Delta W_{IF} = 0$. We also checked that the equilibrium is a convergence-stable strategy, denoted z^*, meaning that if the population is perturbed from the equilibrium then natural selection will push it back $\left(\frac{d\Delta W_{IF}}{dz}\Big|_{z=\hat{z}}\right)$.

We found that the equilibrium of our model, $z^* = 1/(aN)$, is identical to that originally predicted by (Parker and Knowlton, 1980); however, our analysis shows that the optimal territory size strategy is selfish rather than spiteful. Territory size cannot be spiteful in this model because the actor's genetic similarity to individuals in other demes is always equal to or less than the similarity to deme mates ($Q_1 \geq Q_2$). Accordingly, the relatedness to primary recipients (measured relative to secondary recipients) is never negative ($R_1 \geq 0$), and so there is no indirect benefit of larger territory size. Moreover, when offspring dispersal is limited ($m < 1$) and deme mates are positively related ($R_1 > 0$), there is no indirect benefit of smaller territory size (as a form of helping). This is because limited dispersal increases competition among offspring within the deme, which promotes harming and exactly cancels the effect of positive relatedness (Taylor, 1992). Territory size therefore evolves for its direct benefit only, with larger territories promoted by a smaller fecundity cost to the actor (smaller a) and smaller population size (smaller N). Specifically, the direct fitness effect at equilibrium ($z = z^*$) is

$$-C = \frac{aN\,(d-1)\,(1-m)^2}{N-1}, \tag{9}$$

which is either positive (when $m < 1$) or zero (when $m = 1$). In the case of full offspring dispersal ($m = 1$), the equilibrium is the point where the fecundity cost to the actor is exactly balanced by the feedback benefit experienced by its offspring (reduced competition for space in the next generation). As the

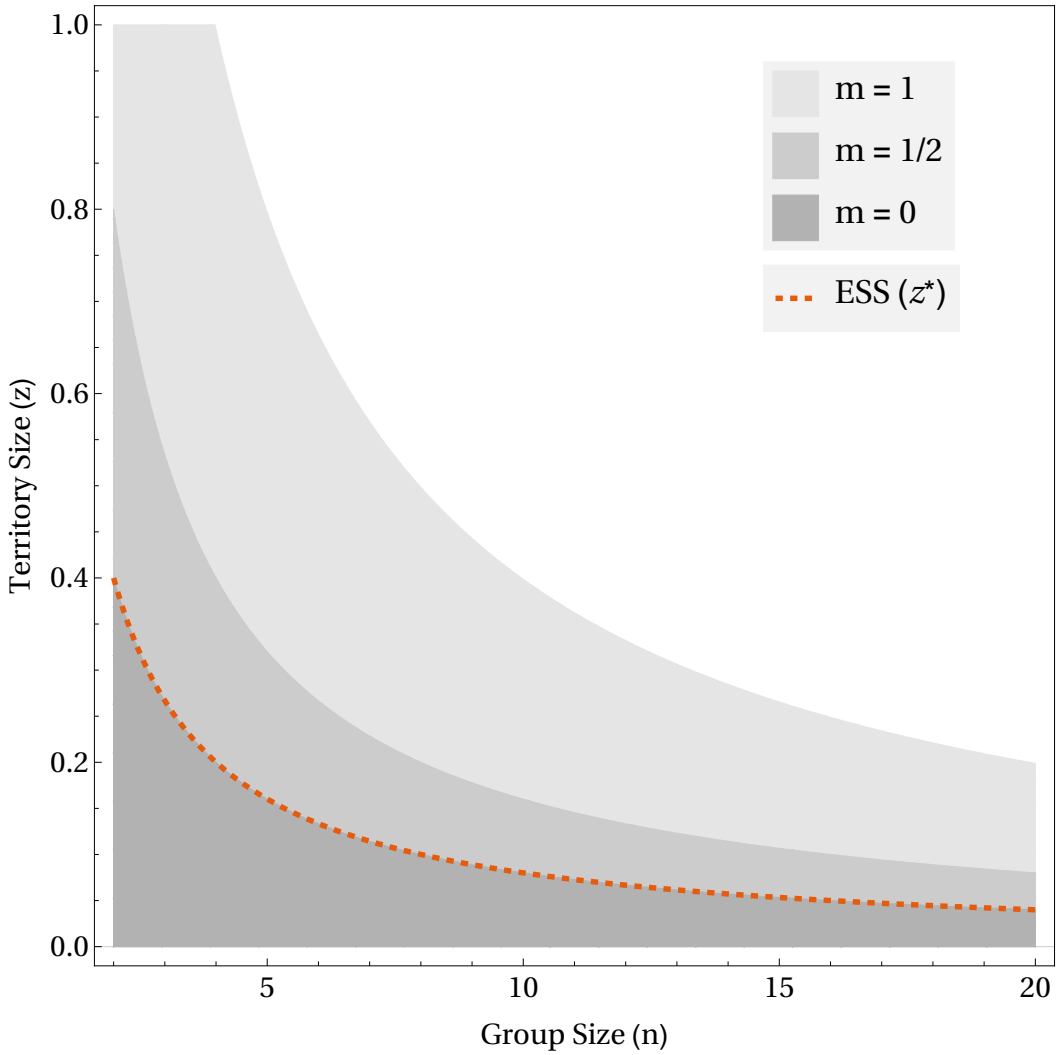

Figure 2: Territory size and direct fitness, the shaded regions indicate where direct fitness is greater than zero for a given migration rate (m). Larger territory size is promoted by smaller population size (smaller dn) and reduced offspring migration from the deme (smaller m), both of which increase the direct benefit to an actor for harming its deme mates. However, reduced migration also increases the relatedness among deme mates, which inhibits larger territory size. Ultimately, the optimal territory size strategy (z^*, dashed line) is independent of migration rate and evolves as if the population were fully mixed ($m = 1$). Other parameters used: $d = 2$, $c = 0.25$.

population approaches this equilibrium, however, direct fitness is always positive ($-C > 0$), confirming that territory size evolves as a selfish trait (fig. 2).

We next assumed that the set of secondary recipients is the entire population, including the focal actor (as in eq. (4)). In this case, the inclusive fitness effect is

$$\begin{aligned}\Delta W_{IF} &= \frac{\partial W}{\partial x} + \frac{\partial W}{\partial y} Q_1 + \frac{\partial W}{\partial z} \bar{Q} \\ &= -c + b_1 Q_1 + b_2 \bar{Q}. \end{aligned} \tag{10}$$

Where z_p is the average territory size strategy in the entire population (including the focal actor), and all partial derivatives are evaluated at $x = y = z_p$. As expected, solving for the equilibrium of eq. (10) gives the same answer as before, $z^* = 1/aN$.

However, we can now see why territory size could be misclassified as spiteful. For example, in a fully mixing population at the equilibrium ($m = 1$; $z_p = z^*$), the first term in eq. (10) is:

$$-c = -\frac{aN}{N-1}, \tag{11}$$

which is always negative. This term reflects the fecundity cost of the focal actor's territory size strategy, however, it is not the total direct fitness effect because it excludes the feedback benefit experienced by the actor's offspring (reduced competition). As noted above, when $m = 1$ this feedback benefit should exactly balance the fecundity cost at equilibrium. Following eq. (5) or eq. (6), we can calculate the feedback benefit as $(-1/(N-1))b_1$ or $(1/N)b_2$ (both evaluated at $z_p = z^*$), which gives the expected result, $aN/(N-1)$. The partitioning in eq. (10) therefore splits the total direct fitness effect of territory size into two separate terms, $-c + (-1/(N-1))b_1$ or $-c + (1/N)b_2$, which could be misinterpreted as a direct fitness cost ($-C < 0$) and an indirect

fitness benefit ($RB > 0$).

4 Discussion

We examined a general model of harming traits and a specific model where larger territory size is an indiscriminate harming trait. In both models we found that: (1) the evolution of spite requires kin discrimination; (2) without kin discrimination, harming can be favoured but only when there is a sufficient direct, feedback benefit to the actor (reduced competition); (3) indiscriminate harming can be favoured most in small populations (or small economic neighbourhoods), where the feedback benefit to the actor is greatest; (4) previous studies have misclassified indiscriminate harming as spite, partly because they misinterpret the feedback benefit as an indirect (kin-selected) benefit ($RB > 0$). Overall, our results support the hypothesis that indiscriminate harming traits are selfish rather than spiteful.

4.1 Classifying harming traits

For the purposes of classifying harming traits, we found that it is easiest to treat the actor, primary recipients, and secondary recipients as separate categories. This makes it straightforward to separate the total direct and indirect fitness effects of harming ($-C$ and RB, respectively) and ensures that non-zero relatedness will always be associated with an indirect fitness effect. For example, spiteful harming ($-C < 0$, $B < 0$) requires that harm is directed at primary recipients to whom the actor is negatively related (with respect to secondary recipients; $Q_1 < Q_2$ and $R_1 < 0$), resulting in a positive indirect fitness effect ($R_1 B > 0$) (Lehmann et al., 2006). In contrast, when harming is indiscriminate, the actor has zero relatedness to primary recipients (with respect to secondary recipients;$Q_1 = Q_2$ and $R_1 = 0$), meaning that harming can be favoured as a selfish trait only ($-C > 0$, $B < 0$).

We showed that misclassification of indiscriminate harming is due to an implicit assumption that the focal actor is a secondary recipient of its own behaviour (Hamilton, 1970; Grafen, 1985; Vickery et al., 2003; Smead and Forber, 2012). This means that some of the actor's direct benefit of harming has been accounted for by a fraction of the fitness effects on recipients, giving the appearance of an indirect benefit ($RB > 0$). For example, in a well-mixed population where all individuals (including the actor) are considered secondary recipients, a fraction of the fitness effect on primary recipients ($-1/(N-1)B_1$) actually contributes to the direct benefit of indiscriminate harming.

Others have suggested that harming traits should be classified based on their primary effects only, rather than their total fitness effects (Krupp, 2013). This means that indiscriminate harming traits like larger territory size, which may be associated with a survival or fecundity cost ($-c < 0$ in the terms of our model), would be classified as spiteful, despite the feedback benefit to the focal actor. We argue, however, that a classification based on total fitness effects ($-C$ and RB) is more useful (Hamilton, 1964; West et al., 2007). This is because it emphasises the fundamental distinction between spiteful harming, which is favoured by indirect fitness benefits and requires kin discrimination, versus selfish harming, which is favoured by direct fitness benefits and does not require kin discrimination (West and Gardner, 2010). Similar arguments have been made for maintaining the distinction between altruistic helping ($-C < 0$, $B > 0$) and mutually-beneficial helping ($-C > 0$, $B > 0$) (West et al., 2007).

4.2 Indiscriminate harming in nature

We found that selfish indiscriminate harming can be favoured most in small populations or small economic neighbourhoods (e.g., small groups with relatively local competition). This is because harming primary recipients leads

to reduced competition for all individuals in the population or group, and a focal actor receives a larger fraction of this secondary benefit when it makes up a larger fraction of the population or group. Indiscriminate harming can therefore be thought of as producing a type of public good for secondary recipients (Tullock, 1979), analogous to indiscriminate helping, which is often thought of as a public good for primary recipients. A key difference is that indiscriminate helping is inhibited by local competition (Taylor, 1992; Griffin et al., 2004); in contrast, indiscriminate harming requires local competition so that the focal actor can actually benefit from the reduced competition that results from its harming (Gardner et al., 2004).

So where can we expect to find indiscriminate harming in nature? As recognised by Hamilton (1970), very small populations will tend to extinction, so harming traits in these populations are unlikely to be observed. An alternative may be small groups with relatively local competition, such that harming an individual reduces competition for local resources. One potential example is in fig wasps, where males fight for access to females, and the intensity of fighting increases sharply as the number of males in the fig declines (Reinhold, 2003; West et al., 2001). Further potential examples include competition among female honey bees for a colony and other cases where males engage in local competition for mates (e.g., *Melittobia* parasitoids; West (2002)).

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A Territory Size model

Here, we derive an expression for the fitness of a focal actor with a mutant territory size strategy, based on the models of Knowlton and Parker (1979); Parker and Knowlton (1980). We consider a population that is structured into d demes of n individuals competing for territories, where each deme has A units of available territory. The focal actor's strategy, x, represents a continuous number of territory units that it attempts to gain ($x > 0$). The average strategy of the actor's deme mates is y, and the average strategy in all other demes is z.

We first calculate the expected offspring production (expected fecundity, F) for the focal actor, an individual in the actor's deme, and an individual in another deme. These expected values depend on: (1) the probability

of an individual acquiring a territory (assuming that available spaces are acquired completely randomly); (2) the cost associated with the individual's strategy (assuming fecundity declines linearly with increasing territory size strategy; $f(x) = 1 - ax$, where $0 < a < 1$). For the focal actor, there are A/y spaces available in the deme, and we use the simplifying assumption that a mutant individual has priority to claim the territory units denoted by its strategy (Knowlton and Parker, 1979). Therefore, the focal actor has a $1/n$ probability of acquiring a territory, and its expected fecundity is:

$$F_x = \frac{A}{y}\frac{1}{n}f(x). \tag{A.1}$$

The space available for others in the patch depends on whether or not the focal actor claims a territory. The actor gains access to the patch with probability A/ny, and in this case $(A - x)/y$ spaces remain; otherwise, A/y spaces are available. The expected fecundity for one of the $n - 1$ deme mates of the focal actor is therefore:

$$F_y = \left(\frac{A}{ny}\frac{A-x}{y}f(y) + \left(1 - \frac{A}{ny}\right)\frac{A}{y}f(y)\right)(n-1)^{-1}. \tag{A.2}$$

Finally, for an individual in another deme in the population, there are A/z spaces available, and so the expected fecundity for one of these individuals is:

$$F_z = \frac{A}{z}\frac{1}{n}f(z), \tag{A.3}$$

We next calculate the focal actor's fitness, ω, which is the number of its offspring that survive to compete for a territory in the next generation. This can be partitioned into two terms, the first term accounting for offspring that compete on the focal actor's natal deme (those that did not disperse, with probability $1 - m$, and those that dispersed but landed on the natal

deme, with probability m/d) and the second term accounting for offspring that disperse with probability m to compete in the $d-1$ non-natal demes:

$$\omega = \frac{\left(1-m+\frac{m}{d}\right)F_x n}{(1-m)F_x+(n-1)(1-m)F_y+\frac{1}{d}(mF_x+(n-1)mF_y)+\frac{d-1}{d}nmF_z}+ \frac{\frac{d-1}{d}nmF_x}{(1-m)nF_z+\frac{1}{d}(mF_x+(n-1)mF_y)+\frac{d-1}{d}nmF_z}. \quad \text{(A.4)}$$

where, the denominator of the first and second terms account for, respectively, all offspring competing in the focal actor's natal deme and all offspring competing in any other deme in the population. Equation (A.4) is the fitness function used to calculate the inclusive fitness effect in eq. (8) of the main text. To express the focal individual's fitness in terms of x, y, and z_p (the average territory size strategy in the entire population, including the focal individual), we substituted $(x+(n-1)y-dnz_p)/(n-nd)$ for z in eq. (A.4). This gives the fitness function used to calculate the inclusive fitness effect in eq. (9) of the main text.

B Deriving probabilities of genetic identity

Here, we derive probabilities of genetic identity by descent in a finite deme-structured population, following the approach of (Taylor et al., 2000). In particular, we needed the probability of identity between the focal actor and a randomly selected deme mate (Q_1), between the actor and a randomly selected individual in another deme (Q_2), and between the actor and a randomly selected individual in the entire population (including itself, Q_2^p), defined as:

$$Q_2^p = \frac{1}{d}\left(\frac{1}{n}+\frac{(n-1)}{n}Q_1\right)+\frac{d-1}{d}Q_2. \quad \text{(B.1)}$$

This is distinct from equation A.1 in Taylor et al. (2000) in that we separate out the individual from the group using the term $\frac{1}{n}+\frac{(n-1)}{n}Q_1$ which in Taylor et al. (2000) would be just Q_1. The above yields the recursions:

$$Q_1 = \left((1-m)^2\left(\frac{1}{n}+\frac{n-1}{n}Q_1\right)+\left(1-(1-m)^2\right)Q_2^p\right)(1-w)^2 \tag{B.2}$$

$$Q_2 = \left((1-m)^2 Q_2+\left(1-(1-m)^2\right)Q_2^p\right)(1-w)^2, \tag{B.3}$$

where, w is the mutation rate. We solve eqs. (B.1) to (B.3) simultaneously and take the Taylor expansion to the first order around $w = 0$ dropping second order and higher terms due to the assumption of weak selection, giving:

$$Q_1 = 1-2dnw, \tag{B.4}$$

$$Q_2 = 1+\left(\frac{2d(m-1)^2}{(m-2)m}-2dn\right)w, \tag{B.5}$$

$$Q_2^p = 1+\frac{2(d(1-(m-2)m(n-1))-1)}{(m-2)m}w. \tag{B.6}$$

These are used in eqs. (8) and (10) of the main text. Therefore in our case the appropriate relatedness would be:

$$R_1 = \frac{Q_1-Q_2}{1-Q_2} = \frac{(1-m)^2}{1+m(2-m)(n-1)} \tag{B.7}$$

5

BACTERIOCINS AND THE EVOLUTION OF BACTERIAL SYMBIOSES

Bacteriocins and the evolution of bacterial symbioses

Matishalin Patel, Stuart West

1 Abstract

Cooperative symbionts enable their hosts to exploit a diversity of environments. A low genetic diversity (high relatedness) between the symbionts within a host is thought to favour cooperation by reducing conflict within the host. However, hosts will not be favoured to transmit their symbionts in costly ways that increase relatedness, unless this also provides an immediate fitness benefit to the host. We suggest that antimicrobial warfare, with compounds such as bacteriocins, could provide a relatively universal reason for why hosts would gain a benefit from increasing the relatedness between bacterial symbionts. We theoretically test this hypothesis with a simple illustrative model that examines whether hosts should manipulate relatedness, and an individual based simulation, where hosts and symbionts can coevolve. We find that hosts can be favoured to manipulate relatedness, to reduce conflict between symbionts, and that this can in turn, select for more cooperative symbionts

keywords:

virulence, cooperation, game theory, kin-selection, evolution, social evolution

2 Introduction

Symbiotic cooperation between species allows new niches to be exploited, and the evolution of more complex life (Bourke, 2011; Douglas, 2014). Aphids can grow feeding only on nutrient poor plant sap, because their symbiotic bacteria provide essential amino acids (Baumann et al., 1995). The siboglinid worms depend upon symbiotic methane or sulphides eating bacteria to survive near hydrothermal vents (Thornhill et al., 2008). Symbiosis between an archeal host cell and an aerobic bacterium gave rise to the eukaryotes.

Both theory and empirical data have suggested that a high genetic relatedness between the symbionts within a host can play a key role in promoting symbiotic cooperation. When symbionts are more closely related, any benefits received from increasing host growth are more likely to be shared with relatives, which increases the kin selected benefit of providing help to hosts (Frank, 1994*a*,*b*; West, Kiers, Simms and Denison, 2002; Foster and Wenseleers, 2006; Leeks et al., 2019). Consistent with this prediction, across different symbioses, bacterial symbionts appear to provide more help to their host when relatedness is higher (Fisher et al., 2017). Additionally, experimental and observational studies have shown that conditions which lead to a higher relatedness also lead to cooperation (Sachs and Wilcox, 2006; Dubilier et al., 2008; Bennett and Moran, 2015).

However, Frank (1996) pointed out that although a higher relatedness would favour symbionts to become more cooperative, hosts would not necessarily be selected to transmit or house their symbionts in ways that increased relatedness. The reason for this is that, assuming cooperation was not adjusted conditionally in response to the local relatedness, symbionts would only evolve a higher level of cooperation in response to relatedness over time. Consequently, all else being equal, there would be no immediate fitness benefit to transmitting symbionts in a way that increased related-

ness.

We suggest that competition through compounds such as bacteriocins could provide a relatively general explanation for why hosts would be selected to increase the relatedness between bacterial symbionts. Recent empirical work has shown that bacteria conditionally up-regulate their production of bacteriocins in response to the presence of competing strains (Majeed et al., 2011; Mavridou et al., 2018; Bhattacharya et al., 2018). Consequently, when there are more competing lineages and so relatedness between interacting bacterial symbionts is lower, bacterial symbionts will put more resources into killing each other. This increased toxin production will reduce bacterial growth, such that the bacteria will be less beneficial to their hosts. We suggest that hosts will be selected to transmit or house their symbionts in ways that increased relatedness, to decrease this costly conflict. We theoretically model the plausibility of this hypothesis, and determine the conditions under which it would most be favoured.

We first develop a deliberately simple model, which captures our hypothesis in an easily interpretable way, which could be applied to many cooperative mutualisms. We then perform a set of simulations of some simple scenarios to test our model with less restrictive assumptions.

3 Models

3.1 Illustrative Model

We assume that symbiotic bacteria inhabit a host. Empirical data has shown that when bacteria encounter competing strains, they increase their production of bacteriocins (Majeed et al., 2011; Mavridou et al., 2018; Bhattacharya et al., 2018). Therefore, when more bacterial strains infect a host, and so the genetic relatedness between symbionts is lower, the growth rate of each strain will be reduced for two reasons: (1) they will be investing more re-

sources into bacteriocin production; (2) the mortality caused by bacteriocins from other strains will be greater. We capture these effects by assuming that symbiont growth is a positive function of the relatedness within the group:

$$S(r) \propto r^{\alpha}, \quad \alpha \in \mathbb{R}_{>0}. \tag{1}$$

The parameter α determines whether the relationship is linear ($\alpha = 1$), decelerating ($\alpha < 1$) or accelerating ($\alpha > 1$). This shape parameter captures all the details of how the production and effect of the toxin interact to influence the growth rate of a symbiont.

We assume that hosts gain more benefit from their bacterial symbionts when these symbionts are better able to grow. We ask whether a host would be favoured to invest in a costly manipulation to influence relatedness amongst their symbionts. This manipulative trait ($g \in [0,1]$) could be a behaviour, such as young eating parental faeces or a structural adaptation, such as specialised organs in the host to store and transmit symbionts (Douglas 1989).

We assume the initial relatedness amongst symbionts infecting a host is $r_s \in [0,1]$. This starting relatedness could be due to existing behaviours, structural constraints or transmission routes. We assume that if a host invests an amount $g \in [0,1]$ in a costly manipulation trait that this increases relatedness to some final relatedness ($r_f \in [0,1]$):

$$r_f = r_s + g(1 - r_s). \tag{2}$$

We assume that the cost of investing in the trait that increases symbiont relatedness reduces host fitness by a fraction $(1-g)^{\beta}$, where β is another shape parameter. We assume that the cost of increasing symbiont relatedness and benefits from symbiont growth interact multiplicatively, such that

host fitness (W_H) is:

$$W_H = (1-g)^{\beta}\,(r_s + g(1-r_s))^{\alpha} \tag{3}$$

We aim to find when this costly trait is favoured. To do this we solve for a candidate evolutionarily stable strategy (ESS) amount of manipulation for the host to invest in (g^*). The ESS (g^*) represents the strategy which could not be beaten by any other strategy, and is given by solving for when fitness is maximised; $\frac{dW_H}{dg}\Big|_{g=g^*} = 0$ (Maynard Smith and Price, 1973):

$$g^* = 1 - \frac{b}{(a+b)(1-r_s)}. \tag{4}$$

Equation (4) predicts hosts can be favoured to perform a costly trait that increases relatedness between symbionts, to reduce the extent to which symbionts invest in costly conflict. Specifically, the ESS host manipulation of relatedness (g^*) will increase when: (i) the starting relatedness amongst symbionts decreases (r_s), (ii) the influence of relatedness on symbiont growth is more accelerating (higher α), and (iii) the cost of manipulating relatedness is more decelerating (lower β).

3.2 Simulation

Our analytical model made a number of simplifying assumptions. In particular, we: (1) assumed a simple relationship between symbiont growth and host fitness; (2) did not specifically model symbiont cooperation, or examine how this would respond to host manipulation; (3) did not allow for coevolution between host and symbiont traits. We explored the consequences of relaxing these assumptions with an individual-based simulation.

We performed two simulations. In the first simulation the symbionts unconditionally express bacteriocins into the environment, however these toxins did not target self. This means that when a host increases relatedness

between bacteria the toxins they produce do not affect each other and the group has a higher average growth rate.

This unconditional response is a simplification, though some species may pursue this strategy as a response to high levels of competition (Bhattacharya et al., 2018). In the second set of simulations we add in a conditional response on the part of the bacteria so that bacteria invest less in bacteriocin when they interact with member's of their own strain. This means relatedness has an effect similar to the first model in that bacteriocin cannot kill other individuals but also there is an extra benefit to the symbiont in that it can dedicate more energy to growth. This model also includes a host quality measure which decreases the more bacteriocin is produced. This models how bacteriocin production might harm the symbionts vertical transmission through reduced host quality.

3.2.1 Simulation with unconditional responses

We assume a population of N hosts in a birth-death Moran process (Zukewich et al., 2013). Each generation there are N individual reproduction events where one member of the host population copies itself, with a probability proportional to its fitness, and another member dies at random.

Each time a host is born, it acquires k symbiont lineages (strains or clones), which can be acquired either vertically from their parent, or horizontally, from the environment. We assume that the host (i) has an evolvable trait m_i that manipulates the likelihood of vertical transmission from its parent, $m \in [0,1]$. Specifically, we assume that the final probability of vertical transmission is given by:

$$\lambda_{f,i} = \lambda_s + m_i(1 - \lambda_s), \tag{5}$$

where, λ_s is the base chance of vertical transmission if the host does not attempt to manipulate it, and $\lambda_{f,i}$ is the final value. This probability is

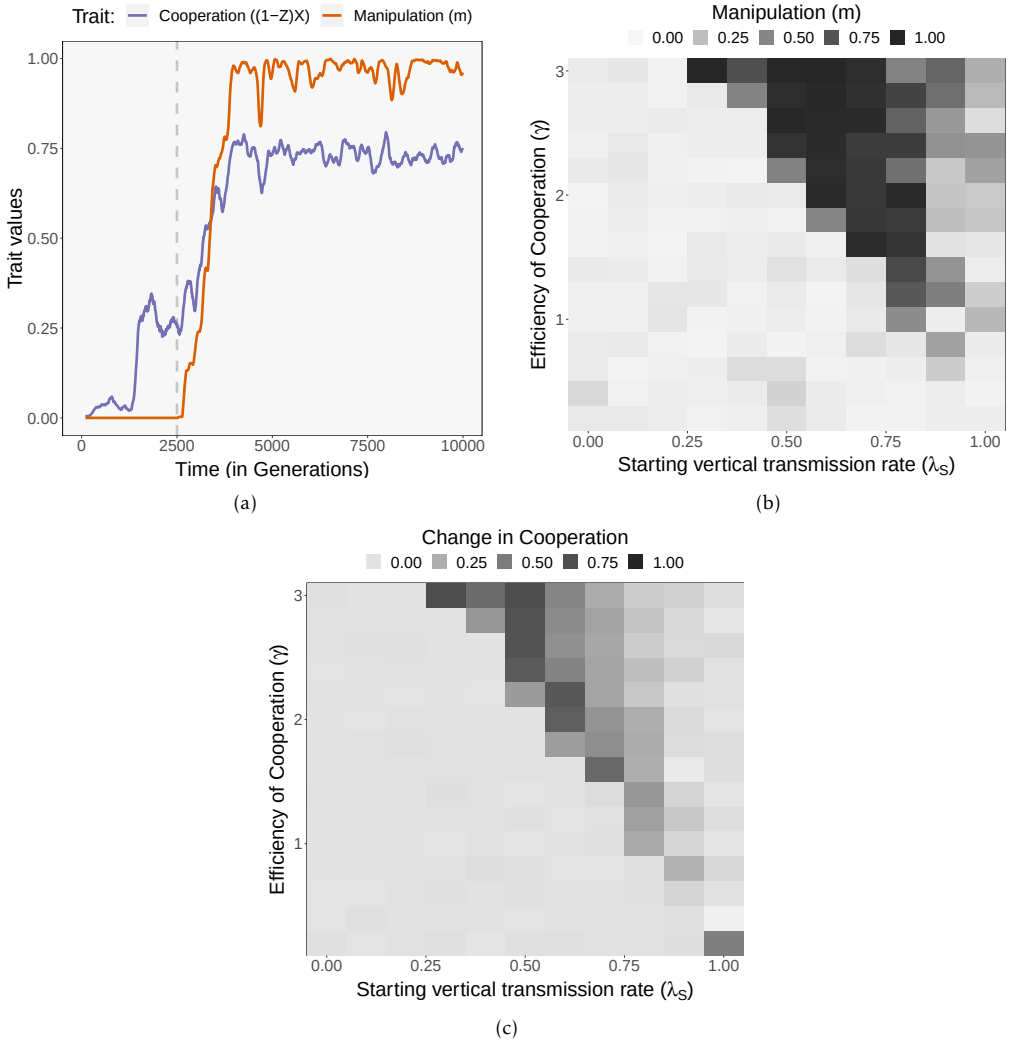


Figure 1: **(a)** An example simulation run with a starting vertical transmission rate of $\lambda_S = 0.5$ and an efficiency of cooperation $a = 2.4$. The gray dashed line indicates the time at which a host can invest in manipulation (orange). A mutant spreads and is followed by an increase in symbiont cooperation (purple). **(b)** Manipulation evolves in the host when starting vertical transmission is intermediate and when efficiency of cooperation is high. When starting relatedness is maximal there is no selection for investing in manipulation. At lower relatednesses manipulation is favoured as long as the benefit from cooperation is great enough. **(c)** Here we measure the change in symbiont cooperation from the symbiont evolved with no host manipulation and the symbiont after manipulation has evolved. In the lower boundary where evolution of manipulation evolves but starting vertical transmission and/or efficiency of cooperation are low is where manipulation has the largest impact on cooperation.

unique to each host based on its personal manipulation trait m_i. After a period of 2500 generations of burn-in the host trait for manipulation m is allowed to evolve from a starting value of 0.

So, with probability $\lambda_{f,i}$ when a host reproduces k symbionts are chosen, with a probability proportional to growth rate relative to the group, from the symbionts within it to colonise its child (vertical transmission). However, with probability $1-\lambda_{f,i}$ those k symbionts are instead chosen from the entire pool of all symbionts within all hosts, with a probability proportional to growth rate relative to the population (horizontal transmission).

We assume that symbionts are under two zero-sum tradeoffs. These are controlled by two evolving traits one for growth (Z) and one for cooperation with the host (X). They may either invest in growth and replication ($Z \in [0,1]$) or the production of extracellular effectors ($1-Z$). Of these extracellular effectors they may either produce a beneficial function for the host ($X \in [0,1]$) or toxins that harm other bacteria ($1-X$). We allow the mutation, and therefore evolution, of Z, X within the symbionts using small deviations drawn from a normal distribution (Norm$(0, 0.1)$), the values are clamped within the interval $[0,1]$.

The relative growth rate of a symbiont will be determined by the amount it invests into growth, and the extent to which it is killed by the bacteriocins of other lineages, relative to the lineages that it is competing with. We assume that toxins are discriminatory and do not target self. We also assume that expression is not conditional (Bhattacharya et al., 2018). This unconditional expression still allows greater growth and less conflict at high relatedness because the toxin's do not target clones. L is the set of all strains (lineages) within a host, within each strain, $l \in L$, there is a set of individuals J. The growth rate ($S_{l',j'}$) for a focal symbiont $j' \in J$ of strain $l' \in L$ inside

host i is given by:

$$S_{l'j'} = Z_{l'j'}\left(1 - \frac{1}{k-1}\sum_{l\in L\setminus l'}\sum_{j\in J}\Big(1-X_{l,j}\Big)\Big(1-Z_{l,j}\Big)\right) \tag{6}$$

This is the amount of energy put into growth by the symbiont ($Z_{l'j'}$) multiplied by the bracketed term indicating the effect of the toxin produced by others. The second term sums over each strain in the host, ignoring individuals of the same strain ($l \in L \setminus l'$), and each individual in that strain. It sums together the contributions from each individual towards toxin production ($(1-X_{l,j})(1-Z_{l,j})$). This entire quantity is then divided by the total number of symbionts (k) in the host excluding the focal individual ($1/(k-1)$) to give the average toxin per capita. This growth rate is calculated for each individual in the group and then divided by the group average to get the relative growth rate of each individual ($W_{S_{l'j'}}$):

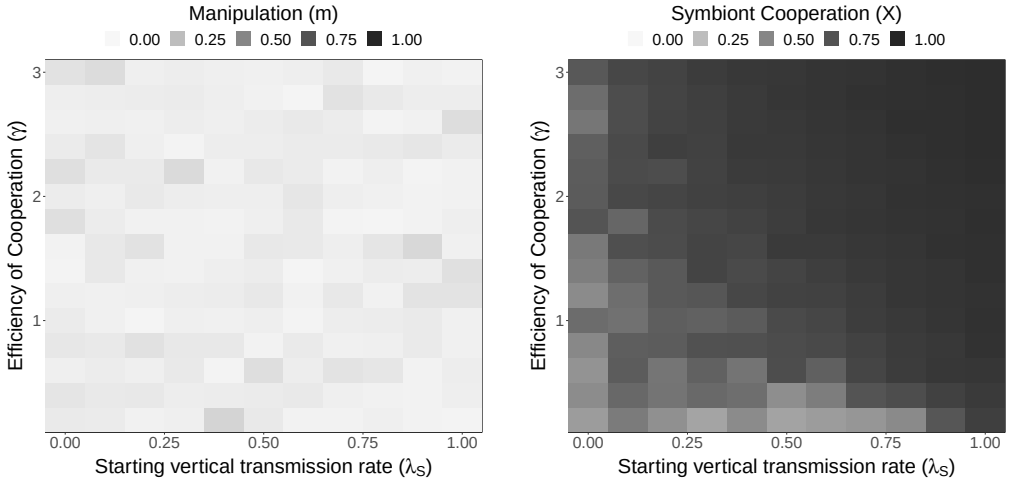
$$W_{S_{l'j'}} = \frac{S_{l'j'}}{\overline{S}} \tag{7}$$

We assume that the fitness of a host (W_H) is given by the sum of the cooperative benefits given by each symbiont weighted by the symbiont relative growth ($W_{S_{l'j'}}$) rate to give the benefit to the host, multiplied by the proportion of resources not invested into symbiont manipulation:

$$W_{H_i} = \left(\sum_{lj} W_{S_{lj}} X_{lj}(1-Z_{lj})\right)^{\gamma}(1-c\,m_i)\,, \tag{8}$$

where, c is the cost per unit of the manipulation trait g, and γ is the efficiency of cooperation.

We simulate populations of hosts using this framework for multiple different parameter sets. Figure 1a shows an example simulation run. After a period of burn-in (2500 generations) a mutant host invades that invests in manipulation. This increase in manipulation leads to an increase in sym-



(a) (b)

Figure 2: **(a)** When the evolution of growth is fixed, $Z = 0.5$, we find that host control doesn't evolve. **(b)** When holding growth at a constant ($Z = 0.5$) cooperation in the symbionts evolves to a high level despite low host control. This leads to no selection for the host to increase control.

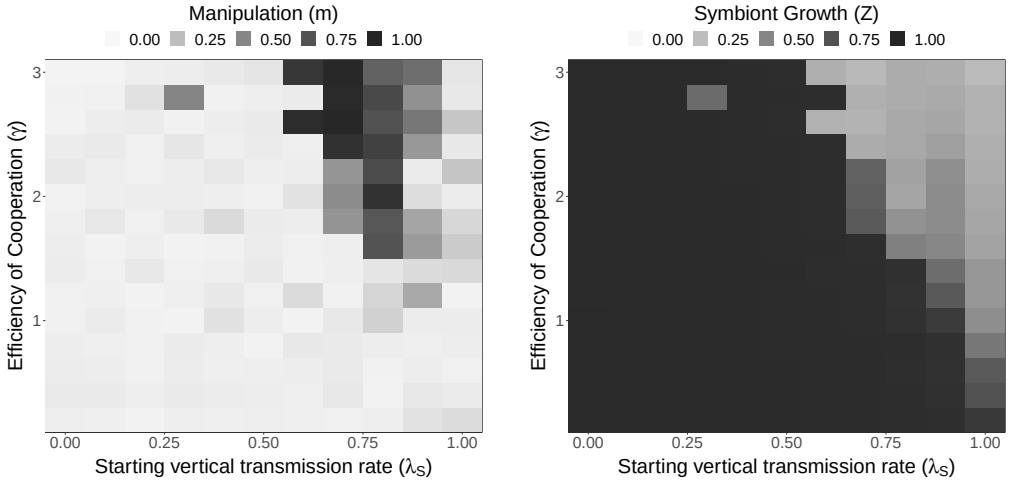
biont cooperation ($(1 - Z)X$).

We found manipulation evolved when the starting vertical transmission rate is at an intermediate value. When starting vertical transmission is too low the increased manipulation seems to not be effective enough and when it is too high symbionts are already highly cooperative so there is no benefit (fig. 1b). Figure 1c shows the difference between this basic level of cooperation and the level of cooperation at the end of a simulation once host manipulation has had an impact. Host manipulation changes symbiont cooperation the most in the same region as it evolves to the highest level.

Holding traits constant We considered three tests to confirm the simulations were acting as we expected. Firstly, when we hold the bacterial growth trait, $Z = 0.5$, constant and allow cooperation X to evolve we observe no evolution of host control (fig. 2). This occurs because holding growth constant leads to the evolution of high levels of cooperation amongst the symbionts ($X \sim 1$) as there is no benefit from bacteriocins when relatedness is low. This leads to no selection for the host to evolve control as there is low conflict regardless. Secondly, when we consider the case where cooperation, $X = 0.5$,

is held constant this leads to limited evolution of host manipulation (fig. 3). When $X = 0.5$ symbionts optimise their growth rate Z to an intermediate value when the starting vertical transmission rate is high and when efficiency of cooperation is high (fig. 3b). Otherwise they evolve a growth rate that is close to one ($Z \sim 1$). When the growth rate is intermediate this increases the production of bacteriocins ($(1 - Z)(1 - X)$). The host can then be selected for to increase relatedness and decrease the level of bacteriocins produced by the symbiont.

Thirdly we show that, when holding both cooperation and growth constant ($X = 0.5$, $Z = 0.5$), control cannot evolve (fig. 4). It may not immediately be clear why this should be the case as our illustrative model shows that manipulation should be stable in this scenario. One possible explanation is that evolutionary divergence between vertical and horizontal symbionts might be necessary to stabilise costly manipulation in our model. Consider a population of symbionts where each member is its own species (maximal conflict). Now a host population that is monomorphic for no manipulation would be invaded by a population that exerts a slight control (á la Taylor and Frank (1996)) as they would experience slightly less conflict. And this process should repeat until an equilibrium is reached, this logic is equivalent to the argument in section 3.1. However, our simulations use a finite population, by increasing relatedness within hosts there is also an increase in relatedness of the horizontally transmitting symbionts (transmission is probabilistic). This leads to the invasion of low manipulation hosts that take advantage of this low symbiont diversity. This exploitation means that manipulation cannot evolve when all symbionts are identical. The ability for symbionts to evolve with their hosts leads to low growth mutants that do not transmit horizontally as well. The feedback in the vertical lineages essentially separates the symbionts into vertical and horizontal pools and reduces the benefit from being a low manipulation mutant as horizontal



(a) (b)

Figure 3: **(a)** When the evolution of cooperation is fixed, $X = 0.5$, we find that host control can evolve. **(b)** When holding cooperation at a constant ($X = 0.5$) selection for maximal growth ($Z = 1$) seem to relax at high vertical transmission rates (λ_s) and high efficiencies (γ). This selects for host mutants that reduce the conflict generated in those regions.

transfer will be dominated by low quality symbionts.

3.2.2 Simulation with conditional response

In the simulations performed in section 3.2.1 we had a discrimination system for the bacteriocin but it was not conditional to the presence of other strains. We also had a relative growth rate effect whereby the toxin produced in a patch only mattered for within host growth and everything was normalised between hosts this may have lead to the earlier simulation underestimating the effect of bacteriocin production when selecting bacteria for horizontal transmission.

To attempt to address this we made two changes to the equations presented in section 3.2.1. Firstly for $S_{l',j'}$ (eq. (6)), which is the growth rates of the symbiont within a host we modified the equation to include a term that describes the amount of toxin that would have been produced by the focal individual but was not. This represents the benefit to the symbiont of not expending energy on bacteriocin production when relatedness is high. This

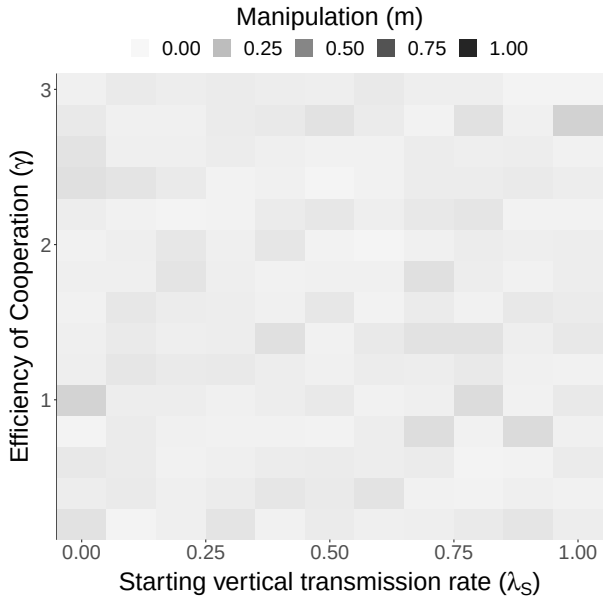


Figure 4: When growth and cooperation are fixed, $Z = 0.5$ and $X = 0.5$, host control does not evolve.

gives a modified symbiont growth rate $S^F_{l'j'}$:

$$S^F_{l'j'} = Z_{l'j'}\left(1 - \frac{1}{k-1}\sum_{l\in L\setminus l'}\sum_{j\in J}\Big(1-X_{l,j}\Big)\Big(1-Z_{l,j}\Big)\right)\cdot \left(1+\sum_{j\in J\setminus j'}\Big(1-X_{l',j}\Big)\Big(1-Z_{l',j}\Big)\right). \tag{9}$$

The third term here is the sum of the toxin that would have been expended for each member of the focal individual's strain. This is then used to calculate a relative growth rate:

$$W^F_{S^F_{l'j'}} = \frac{S^F_{l'j'}}{\overline{S}} \tag{10}$$

The second change was made to the host fitness function, $W^F_{H_i}$:

$$W_{H_i} = \left(\sum_{lj} W^F_{S^F_{lj}} X_{lj}(1-Z_{lj})\right)^{\gamma} \frac{1}{k}\left(1-\sum_{lj}\left(\sum_{l\in L\setminus l'}\sum_{j\in J}\Big(1-X_{l,j}\Big)\Big(1-Z_{l,j}\Big)\right)\right)(1-c\,m_i)\,. \tag{11}$$

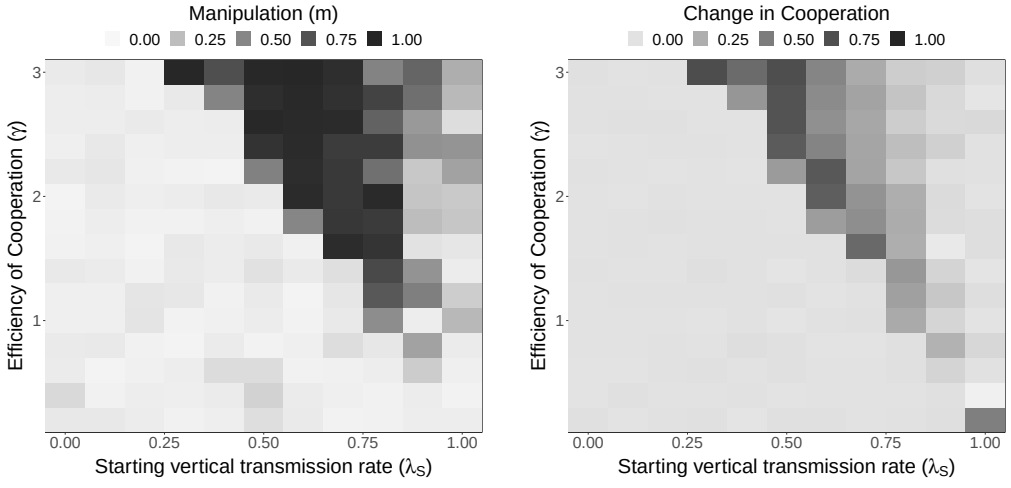


Figure 5: **(a)** Manipulation evolves in the host when starting vertical transmission is intermediate and when efficiency of cooperation is high. The patterns are similar to those found when doing the simulation without the explicit feedbacks (fig. 1b). **(b)** The change in symbiont cooperation from the symbiont evolved with no host manipulation and the symbiont after manipulation has evolved is shown here. In the lower boundary where evolution of manipulation evolves but starting vertical transmission and/or efficiency of cooperation are low is where manipulation has the largest impact on cooperation. This is again similar to the pattern observed before (fig. 1c).

We insert a term after calculating the benefit from the cooperation produced by each symbiont, the new term calculates the amount of toxin produced by each symbiont within the host. This then penalises the cooperative benefit the symbionts given to the host. This captures the idea that host's with high levels of bacteriocin production will have a lower quality than those without as the symbionts will reach a lower carrying capacity within the host.

Figure 5 shows the results of the modified simulations. The pattern is qualitatively identical to before with intermediate vertical transmission rates and high efficiencies of cooperation favouring the evolution of host control.

We also performed the same diagnostic simulations as those done for the first simulation with unconditional expression. We recovered the same patterns for our second simulation model (appendix A.1.1).

4 Discussion

We have shown that: (1) a host can be selected to increase relatedness amongst its bacterial symbionts to decrease conflict via antimicrobials such as bacteriocins; (2) this, in turn, selects for higher levels of cooperation from symbionts towards their hosts.

4.1 Illustrative model

Our illustrative model assumed that higher relatedness increases the growth rate of the symbiont. This is based upon experimental work on density dependent behaviour in bacteriocin production (Bashey et al., 2012; Mavridou et al., 2018). However, previous theory suggests that bacteriocin production may in fact follow a domed relationship with relatedness (Gardner et al., 2004). This would mean that increasing relatedness may decrease growth rates depending on the starting relatedness. This implies that in nature the evolution of host control may be constrained by the un-manipulated relatedness as sometimes selection may favour reducing relatedness. Indeed, we might predict that evolution should favour either highly related symbionts or highly unrelated ones to avoid these intermediate relatedness values where conflict is high.

Our models predict that hosts will invest more resources into increasing relatedness when this will lead to a greater increase in fitness. For example, in systems where relatedness between symbionts would otherwise be very low, due to low rates of vertical transmission, or due to population structuring leads to greater mixing and conflict between different bacterial strains (fig. 1b). The diversity of mutualisms in the natural world would allow our predictions to be tested with across species comparative studies. For example, amongst the mycetocyte symbionts that infect a variety of insects the mode of vertical transmission varies from faecal smearing to specialised structures that transfer the symbionts from the mycetocytes to the ovaries

(Douglas, 1989). Previous empirical work on termites and ants has shown that they can be selected to maintain their fungal symbionts at high relatedness, to reduce incompatibility conflicts (Korb and Aanen, 2003; Poulsen and Boomsma, 2005; Aanen et al., 2009). Our hypothesis provides an analogous mechanism which could operate across all bacterial symbioses.

4.2 Simulations

The simulations agreed with the model in their patterns at the macro-scale. However the fact that holding the symbiont traits constant removed any selective pressure for the evolution of control shows the important dynamics that can be missed by static models. Our analytic model used a simple behavioural rule for the symbiont to show that costly manipulation could be favoured by the host. Our simulations embedded a similar rule into a dynamic system with finite population sizes, and we found that the evolution of manipulation in this scenario could not work when symbionts were all identical. We suggest that manipulation exerted by the hosts leads to a public goods dilemma were the good was the symbiont diversity. By investing in control hosts reduced the diversity for everyone including those who did not invest. Allowing the symbionts to evolve in response breaks this pattern. Evolution would lead to a separation between the symbiont lineages that are transmitted between hosts and within hosts. This separation breaks the advantage for low host manipulation mutants and stabilises manipulation in the host population. This argument for why evolution of control is not stable when symbionts cannot evolve could be tested properly by keeping track of the symbiont lineages during the simulation which was not a feature included in our simulation.

The simulations we used relied on abstraction when it came to most of the symbiont interactions. This was mainly done to spare computational time. Future work could improve on this by modelling the within host dy-

namics of the symbionts more explicitly. Modelling the individual symbiont interactions explicitly would also allow us to more easily keep track of the lineages of symbionts and confirm our suggestion that this bifurcation between strategies is what stabilises host manipulation.

4.3 Conclusion

We have focused on how a shared interest between symbionts and their hosts can favour symbiont cooperation. Analogous predictions could apply to cases where cooperation is enforced, via mechanisms such as sanctions or trading (Kiers et al., 2003, 2011). In those cases, enforcement works best when it is directed at clonal groups, such as within a legume nodule (West, Kiers, Simms and Denison, 2002; West, Kiers, Pen and Denison, 2002; Wyatt et al., 2013). Reducing costly bacteriocin production provides a fitness benefit for hosts to do this, with the knock on influence that it would favour enforcement mechanisms, which would lead to even higher levels of cooperation. Consequently, reducing microbial competition could facilitate symbiont cooperation more generally, irrespective of whether it arises via a shared interest or enforcement.

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A Appendix

A.1 Simulations

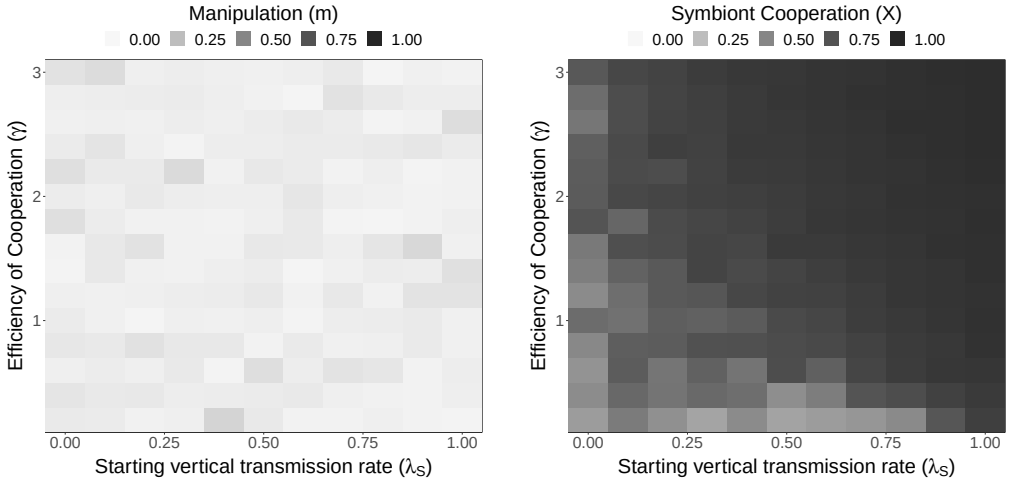
Our individual based simulations are composed of two types of individuals: hosts and symbionts. Each simulation has a fixed number of hosts (N) and a fixed number of symbionts per host (k). Each generation there are N individual reproduction events where one member of the host population copies itself, with a probability proportional to its fitness, and another member dies at random. The order in which this takes place is randomised to avoid biasing the model to the evolution of certain types of traits (Zukewich et al., 2013).

The fitnesses of the newly created symbionts and the new host are updated using the equations for fitnesses in the main text (eqs. (7) and (8)). Then a new host is chosen and the cycle repeats. By performing a host replacement N times we simulate one generation. Simulations were run for 10000 generations, for the first 2500 generations the host's manipulations trait was not allowed to evolve to allow the symbionts to reach an equilibrium.

The simulations used in this paper were done using a population size of $N = 200$ hosts and $k = 5$ symbionts per host. The cost for the manipulation was kept low but significant at $c = 0.1$. Each simulation was replicated 5 times.

Simulation code was written in the julia programming language version 1.1 and run locally on a Dell Optiplex 7040 computer running Ubuntu 17.10. The starting vertical transmission (λ_S) was sampled in the interval $[0, 1]$ in 0.1 increments. The shape parameter for cooperation (α) was sampled in the interval $[0.2, 3]$ in 0.2 increments.

This sampling gave 165 different combinations which were each simulated over 5 repeats, leading to 825 observations.

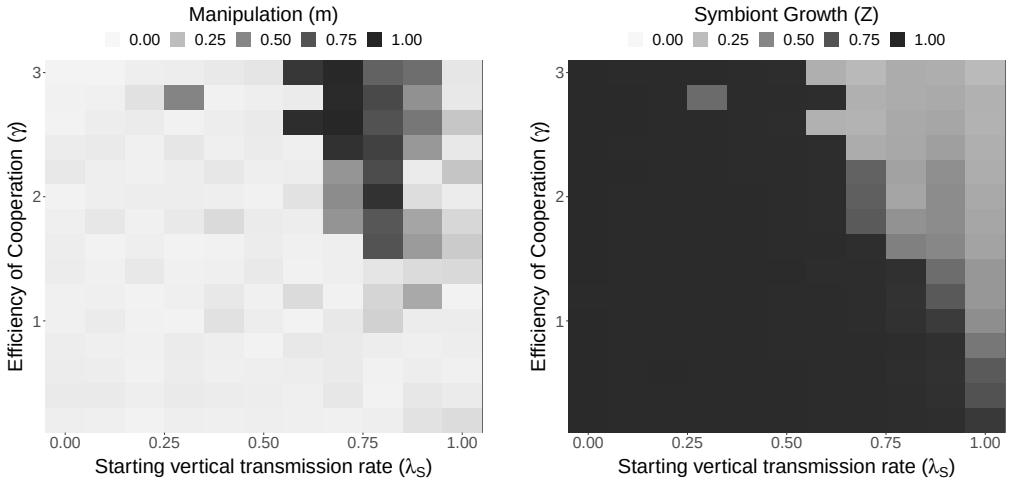


(a) (b)

Figure A.1: **(a)** When the evolution of growth is fixed, $Z = 0.5$, we find that host control doesn't evolve in the modified model with feedbacks. **(b)** When holding growth at a constant ($Z = 0.5$) cooperation in the symbionts evolves to a high level despite low host control. This again leads to no selection for the host to increase control.

A.1.1 Conditional expression plots

We find grossly the same behaviour in the second simulation as in the first. When growth is held constant ($Z = 0.5$) and cooperation (X) is allowed to evolve then investment into bacteriocins is minimal and so host control has no selective advantage (fig. A.1). When instead cooperation is held constant and growth is allowed to evolve ($X = 0.5$) then we find that growth evolves to be lower at high rates of vertical transmission and when cooperation is efficient this leads to host manipulation evolving to reduce the associated increase in bacteriocin production (fig. A.2). Finally when both are fixed we again see no host control evolving. Which is due to manipulation of relatedness not leading to a separation of symbiont pools and therefore benefits cannot be restricted to manipulating hosts which leads to host manipulation being unstable (fig. A.3).



(a) (b)

Figure A.2: **(a)**Similar to what was found in the first simulation with no feedbacks when the evolution of cooperation is fixed, $X = 0.5$, we find that host control can evolve. **(b)** When holding cooperation at a constant ($X = 0.5$) selection for maximal growth ($Z = 1$) seem to relax at high vertical transmission rates (λ_s) and high efficiencies (γ). This selects for host mutants that reduce the conflict generated in those regions.

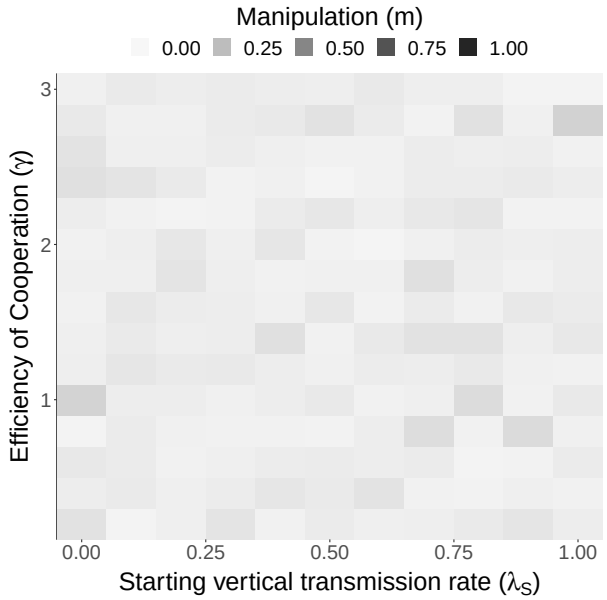


Figure A.3: For the feedback model we again see that when growth and cooperation are fixed, $Z = 0.5$ and $X = 0.5$, host control does not evolve in the parameters explored.

6

DISCUSSION

As an integrated thesis each chapter is a self-contained element and therefore includes its own discussion. In this chapter I bring together some thoughts that emerge from viewing these chapters as a whole. I also discuss some future direction this work could take.

6.1 THE VOLUNTEER'S DILEMMA AND ECO-EVOLUTIONARY FEEDBACKS

In chapter 2 I show how the growth and virulence of the bacteria *Bacillus thuringiensis* depends on a social game — the production of Crystal toxins. The production of toxin is a form of social game called a volunteer's dilemma where some members of a group must volunteer to produce a benefit to all members of the group (including themselves)(Höfte and Whiteley, 1989; Raymond and Bonsall, 2013). The benefit from these kinds of goods are highly nonlinear; once the good has been produced there is no additional benefit for over-producing, and any amount of good lower than the threshold gives no benefit at all (Archetti, 2009). The return on investment is then often like a step function. I showed how this non-linearity introduces strong density and frequency dependent effects between cooperators (who pro-

duce the crystal toxins) and defectors (who do not), even in well mixed populations. I also presented in this paper a way to simplify aggregation into these kinds of multiplayer games using a modified Poisson Binomial distribution. The volunteer's dilemma is a very widely applicable game to biological scenarios. Quorum sensing and costly signals in general fall under its umbrella (Darch et al., 2012).

If volunteer's dilemmas are more widespread in social interactions the fact that they are under these strong density and frequency dependent effects could lead to significant eco-evolutionary feedbacks. In chapter 3 I investigated this idea further by attempting to explicitly include these ecological factors as well as the evolutionary ones (Ross-Gillespie et al., 2007; Ross-Gillespie et al., 2009; Sanchez and Gore, 2013; Gokhale and Hauert, 2016). I showed that over long timescales the evolutionary and ecological dynamics could interact and lead to intermittent fluctuations in both the evolutionary (frequency of defectors) and ecological (population densities) signals of a population.

My simulations in chapter 3 show that the short term within-year dynamics when mapped year to year lead to these fluctuations. However, the methods I used to develop and understand these models were not suited to analysing these kinds of chaotic dynamics. I think the next step in this work is to establish whether the dynamics are as chaotic as they look and if I can detect exactly what the critical parameters are that change the mapping from a predictable deterministic one to a chaotic deterministic map. Some of the analysis that has been done on

transient population dynamics could prove useful (Stott, 2016). These perturbation analysis methods have focused on single species with multiple classes so it is unclear how much more difficult it would be to extend it into more than one species. There has been some similar work in bacterial phage dynamics however the host dynamics are most often held constant in all the analytical methods to make them tractable (Gandon, 2016; Gandon et al., 2016).

Both chapter 2 and chapter 3 use a simplified form of relatedness and group aggregation using draws from a Poisson Binomial distribution. This enforces a certain form of sequential group formation, which is generalisable but could be quite biasing. In nature quantities such as aggregation are process driven not in built parameters. Extending this work to more explicit spatial model could give a better understanding of how aggregation (relatedness) changes given these strong interactions between disease ecology and evolution.

6.2 SPITE AND RELATIVES

In chapter 4 I explore the ideas of indiscriminate spite that have been around since Hamilton (1970). When talking about social behaviour one easily gets lost in a morass of causal chains and fuzzy definitions. In chapter 4 I attempted clarify the classification of spite using an inclusive fitness framework with two parties. Using this methodology I determined that indiscriminate spite as proposed by previous studies underestimated the

size of the direct fitness benefit (Knowlton and Parker, 1979; Parker and Knowlton, 1980; Vickery et al., 2003). By using a more well defined measure of relatedness for finite populations we found that the spite earlier studies described should actually be considered indiscriminate harming as there is a selfish benefit.

There is an important distinction here between discriminate and indiscriminate actions. It is often assumed that discrimination is the act of choosing a subset of individuals for an action that could affect more than that subset, whereas indiscriminate actions are those that target all individuals equally. This distinction may appear clear but it is worthwhile to consider whether not affecting yourself is discriminatory? It could be argued that traits such as territory size are discriminatory to kin because an individual that increases its territory size cannot by definition also be decreasing its territory size. The actor cannot be affected by the trait it expresses. Is this discrimination? I would argue it is not but this view is dependent on viewing discrimination of self as an evident property of existing. A gene need not wonder if it exists — *evolutio ergo sum*. If however, we view discrimination as strictly the exclusion of some targets over others then one could argue that some traits discriminate via the action of the individual and some are inherently discriminatory.

I do not think the maths would change if I were to consider an indiscriminate trait that could affect self, it would require a much larger benefit to compensate for the dual costs of doing the action and bearing the negative benefit. However, the distinction is important when considering indiscriminate spite in nature.

We might expect that harming will be much more common when the harming trait is inherently self-discriminatory.

6.3 HARMING AND HOST MANIPULATION

In chapter 5 I consider a form of spite which is highly discriminatory. In this chapter I was interested in the idea that bacteriocin production between bacterial strains (discriminate spite) could be a driver for the host to invest in increasing relatedness amongst its symbionts. Frank (1994, 1996) showed that there must be an immediate benefit to the host of increasing relatedness, otherwise the mutant host that did so would not see any benefit in its lifetime and would not spread. Bacteriocins that target non-kin are prevalent in many bacterial species (Riley and Wertz, 2002; Mavridou et al., 2018; Granato et al., 2019). These toxins reduce the growth rate of competing strains and can be unconditionally or conditionally expressed (Bashey et al., 2012; Bhattacharya et al., 2018; Mavridou et al., 2018). It seems reasonable then for a host carrying many bacterial strains to gain an immediate benefit for increasing relatedness as it would reduce the inhibitory effect of the toxin's on the symbiont growth.

I showed that in principle this could work using a simple model and slightly more realistic simulation. One initially confusing result that came from our simulation was the fact that if bacterial traits were not allowed to evolve then the host could not evolve a stable level of manipulation. My suggestion for why

this is the case is that because the populations are small and finite in our simulations evolving a low level of manipulation increases relatedness not only within the host but also in the environment. This disincentivises investment in manipulation, and mutants that do not invest in manipulation take advantage of this higher environmental relatedness and spread. When symbionts can evolve however they trade-off horizontal transfer ability for better vertical transmission. This trade-off leads to the isolation of within host and without host symbiont lineages therefore the host mutant that evolve a lower relatedness takes in low quality horizontally transferring symbionts. This penalises the mutant for taking in horizontal symbionts and stabilises a high level of manipulation by the host.

While the chain of logic is attractive, and some initial tests back up the idea, the simulation I ran did not collect the right data to say definitively if this is why the host manipulation fails. I would like to more explicitly test this idea before publication, by keeping track of symbionts as they transmit between and within hosts more closely.

6.4 CONCLUDING REMARKS

Evolution by natural selection has led to a wide diversity of complex social interactions. Understanding these interactions in their fullness can be daunting. In my work, I have tried to attack small parts of these overarching questions using the elegant mathematical tools that evolutionary theory has provided

(Hamilton, 1964; Price, 1970; Queller, 1992; Taylor and Frank, 1996).

The importance of feedbacks between ecology and evolution is unquestionable but trying to understand these kinds of dynamics often leads to intractable equations. By understanding small slices and resorting to exploratory simulations I believe understanding these processes more clearly is possible.

The evolution of symbiosis represent some of the more extreme examples of adaptation in the natural world. Understanding these systems is not only useful for its own sake but I believe more widely useful when considering the symbioses we might wish to engineer or engender using modern technology.

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DECLARATION

I declare that the work herein has been written and collated by me except where stated in the declarations of authorship. This work has not been submitted for any other qualification, in this University or elsewhere.

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