The impact of predation on the evolution of hosts and parasites.

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Abstract

The study of infectious diseases, particularly those transmitted by parasites, is vital to improving our understanding of the evolutionary and population dynamics which may arise during an epidemic outbreak or ongoing endemic. The evolution of host-parasite systems within complex communities is not fully understood. With the use of mathematical models, I build upon our theoretical knowledge of this area. By including a predator population in these systems, I determine how this impacts the evolution of host resistance against infection and multiple enemies, specifically parasites and predators. I also determine when parasite populations are likely to evolve intermediate host manipulation in order to reach a final host. Additionally, trade-offs are included within the host and parasite populations as evolving such defences or manipulation is likely to come at a cost. The presence of the predator increases the potential for host diversity to arise. Experimental work is used to determine the existence of trade-offs occurring in the *Plodia interpunctella* (Indian meal moth) host system. My results reveal the underlying ecological feedbacks, created by relative population densities and growth rates, which drive host-parasite evolution, whilst uncovering the key effects of the predator population. The inclusion of such complexities is important as they clearly affect host-parasite evolution. Overall, I provide an insight into the interesting dynamics arising in these systems. Future studies in this area will continue to develop and improve our understanding of these complex systems.

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Chapter 1

Introduction

1.1 Background and motivation

Research on infectious diseases is important and by modelling infectious disease dynamics, we are able to develop our insights in this field. Given the recent outbreaks of epidemics, such as the Zika and Ebola viruses, as well as ongoing endemic diseases, such as neglected tropical diseases and malaria, this field is becoming increasingly important. With the use of mathematics, we can model infectious disease dynamics, including the spread of parasite-transmitted diseases, to learn more about the population dynamics and evolution of the host, as well as the parasite, when an outbreak occurs. I have studied the complexity of host-parasite systems, specifically their evolutionary and population dynamics, within ecological communities. Although host-parasite systems have been well studied, wider, complex community interactions, such as predation, have often been overlooked. Clearly we know that host-parasite populations will interact with other populations, such as predators, so I focus upon host-parasite models with the inclusion of a predator population which preys upon the host population to determine how this impacts the evolution of host defence mechanisms and the evolution of host manipulation by parasites. Using mathematical models and experimental

work, I build upon our understanding of the evolution of infectious disease systems.

Classic disease models have been used to model host-parasite populations, in which the hosts move from a susceptible class to an infected class after becoming infected. Previous theoretical work on the evolution of host defences to parasitism with the use of such models has provided us with a deep insight into host-parasite systems. In response to a parasite being present in the environment, the host population may evolve a variety of defence mechanisms, including avoidance which lowers the probability of becoming infected by the parasite, recovery which offers a faster rate of clearance, tolerance which reduces mortality when infected and acquired immunity which offers boosted protection against an infection (Miller et al., 2006). Diversity arising in these systems through disruptive selection resulting in the coexistence of strains with different traits (referred to as evolutionary branching) is also typically studied. Such diversity is known to occur in the host when defence is through resistance, but not when defence is through tolerance (Boots and Bowers, 1999, Roy and Kirchner, 2000, Miller et al., 2005, Best et al., 2008). In my models in Chapters 2 and 3, we assume that host defence occurs through avoidance. Early work by van Baalen (1998) showed that host defence through increased recovery is maximised at intermediate rates of parasite-induced mortality (also referred to as virulence), whilst Boots and Haraguchi (1999) showed that lower host defence occurs for highly virulent, sterilising diseases (i.e. the infected individual can no longer reproduce) due to the reduction in disease prevalence. Additionally, host defence has been shown to be generally low for shorter-lived hosts as they are less likely to become infected, with host defence being highest when mortality is low (Miller et al., 2007). However, the results are complicated when acquired immunity is included (Miller et al., 2007, Best and Hoyle, 2013). More recently, Donnelly et al. (2015) studied various host-parasite interactions finding that maximum host investment in defence occurs at intermediate lifespans for a

range of these interactions. A further review on the evolution of costly host defence against parasites is given by Boots et al. (2009). These studies have given us an insight into the evolution of host defences to parasitism.

Evolution from the parasites' perspective has been also studied theoretically as they are able to use various strategies throughout their life-cycle. Parasites can be transmitted through direct or sexual contacts, carried by vectors or have free-living environmental stages (Day, 2001, Taylor et al., 2001, Pietrock and Marcogliese, 2003). However, not much is known about the evolution of more complicated transmission processes, for example intermediate host manipulation, which may be used by parasites. In terms of parasite diversity, Bremermann and Pickering (1983) showed that a single parasite strain is typically optimal. Although such use of mathematical models of these systems has given us an insight into how the host and parasite populations evolve, they have mostly focussed on the two populations in isolation from other populations which they are likely to interact with in nature.

More recently, other populations, specifically predators, have been taken into account within host-parasite models. The addition of the predator has been shown to impact the evolution of host resistance such that there can be deterministic eradication of the disease, which cannot occur in a standard host-parasite model and it can increase the potential for host diversity to occur (Hoyle et al., 2012). Along with the impact of predation on the host population, recent studies have looked at how this affects the evolution of the parasite. The addition of a predator can lead to the evolution of highly virulent parasites which may ultimately lead to predator extinction (Morozov and Adamson, 2011) or parasite diversity (Morozov and Best, 2012). It can also lead to evolutionary cycles of pathogen virulence and predator density (Kisdi et al., 2013). As this previous work on parasite evolution has mainly focussed on the evolution of virulence, other areas such as the evolution of intermediate host manipulation remain relatively unexplored. This more

complex, two-step transmission process occurs when parasites must first infect an intermediate host before they can reach their final host. In this case, the intermediate host is typically a prey species which may be manipulated by the parasite to act conspicuously thereby increasing its chances of being preyed upon by a predator, the parasites final host. Here, parasite manipulation of the intermediate host has been shown to lead to oscillations in population densities (Fenton and Rands, 2006).

Along with theoretical work on these systems, experimental studies have also been carried out. Selective predation has been found in many populations, including snowshoe hares and red grouse, as once infected they are typically more prone to predation (Hudson et al., 1992, Murray et al., 1997). The evolution of host defence mechanisms against multiple enemies has been studied by Friman and Buckling (2012) as they conducted experimental studies on *Pseudomonas fluorescens* bacteria with phage and protists finding that the bacteria diversified when faced with both enemies. Cases showing evidence of host manipulation by parasites have also been found, for example, ants infected by *Dicrocoelium dendriticum* are manipulated making them more prone to predation by sheep and cattle, the parasites final host (Poulin, 1994). Despite these studies, there is little understanding of the evolutionary dynamics arising for host-parasite models when a predator population is present. In our work, we use host-parasite models with the inclusion of a predator to deepen our understanding of these systems.

To gain further insight into host-parasite evolution within complex communities, I include a predator population and determine how this impacts evolution. I look at how the presence of the predator population affects the evolution of host defence against a disease and the evolution of intermediate host manipulation by parasites. I also determine how the host population decides whether to defend itself against infection or predation, given that it is likely that the host will have limited resources and increasing defence against one enemy could constrain defences against the other. Additionally,

I consider the potential for diversity to arise in the host and parasite populations. Given the complexities of such systems in nature, this is a growing research area and by focussing on these particular areas of interest, with the use of theoretical and experimental work, I am able to contribute to the development of our understanding of host-parasite systems.

1.2 Adaptive dynamics

Whilst I explore the evolutionary dynamics of host resistance and parasite manipulation, I work within the framework of adaptive dynamics (Geritz et al., 1998) to determine how evolution will occur. There are four key assumptions underlying the theory of adaptive dynamics (Geritz et al., 1998, Geritz and Gyllenberg, 2005). Firstly, it assumes that there is asexual reproduction, i.e. there is no mixing of different types within the population and the resulting offspring are phenotypically identical to their parent. Secondly, the phenotypes are assumed to vary continuously. Thirdly, it is assumed that mutations are infrequent such that the population stabilises before a new mutant appears. Fourthly, it assumes that evolution carries out in small, discrete steps so the mutations are small but random, i.e. the mutant and resident phenotypes are similar.

There are alternative methods to studying evolution that have been developed prior to the relatively new theory of adaptive dynamics (Abrams, 2001). The quantitative genetic methods use quantitative genetic equations to study the evolution of mean trait values (Abrams, 2001, Waxman and Gavrilets, 2005). Here the number and the types of possible genotypes are often specified, whereas, in adaptive dynamics, a more general range of phenotypic traits is specified. The evolutionarily stable strategy (ESS) methods study long-term phenotypic evolution when fitness depends on the frequencies of the phenotypes present in the population (Hamilton, 1967, Maynard Smith, 1981, Geritz et al., 1998). However, with this method it is unclear whether

the ESS will actually be reached as the population may or may not evolve towards this point. Hence, adaptive dynamics provides an advantage as it accounts for the evolutionarily stability and considers whether the point will be converged to (known as convergence stability, further described below). Additionally, the fitness equations which inform us of the long-term outcome of evolution within adaptive dynamics are applicable to populations that have density and frequency-dependent terms (Waxman and Gavrilets, 2005). Given that adaptive dynamics is more applicable to studying evolution in my work, it is the method used throughout the following chapters.

Using the theory of adaptive dynamics, I start with a resident strain which I assume is at equilibrium. I then introduce a small, rare, nearby mutant strain and determine if it will invade the resident or not. These mutant strains have varying traits along a trade-off curve, in which an increase in investment in one trait constrains investment in another (see Section 1.3 for more on the trade-off curves). We can change this trade-off depending on the type of evolutionary process we are considering. In terms of host trade-offs, the mutant strains may have a higher birth rate and lower defences against parasitism (Chapter 2) or higher defence against one enemy and lower defences against the other (Chapter 3). In terms of parasite trade-offs, the mutant strains have varying levels of manipulation and spore production, for example, they may have higher manipulation and lower spore production (Chapter 5).

Next, to determine whether a mutant can invade the current resident or not, we use a fitness equation, denoted by s for the host or r for the parasite, which tells us the outcome of this process (refer to Box 1.1 for the computation of fitness equation). Evolutionary singular points occur when the gradient of the fitness equation is zero. If $s, r > 0$, the mutant is able to successfully invade the resident becoming the new resident (mutant has positive fitness) and we then repeat the process to see if it is invaded by another mutant. If $s, r < 0$, the mutant cannot invade and the current

resident remains (mutant has negative fitness). If $s, r = 0$, the mutant and resident have the same phenotype. This continues until we determine the final outcome of this evolutionary process. Thus, we gain a view on how the evolution of the host and parasite populations will occur.

Pairwise invasibility plots (PIPs) are one method used to verify the evolutionary behaviour of the systems studied (shown in Figure 1.1). The PIPs show when a mutant population can invade a resident population which is at equilibrium. In the shaded region, $s, r > 0$, whereas, in the unshaded region, $s, r < 0$. Along the main diagonal, $s, r = 0$. The point where a curve (see in Figure 1.1) intersects the main diagonal is a singular point. The population evolves up or down the main diagonal, i.e. a shift in the singular point along the PIP diagonal corresponds to an increase or decrease in the trait. There are different types of singular points which depend on whether the point is evolutionarily stable (ES) and convergence stable (CS). Using the PIP, we can determine visually the type of singular point that arises. In the PIP, if the vertical line through the singular point lies in a negative region (unshaded), the point is ES (Geritz et al., 1998). This means that the point is an evolutionary trap and once it is reached, the population will stay at this point (no nearby mutant can invade the population). If the region is positive above the diagonal to the left and below the diagonal to the right of the singular point (shaded), the point is CS (Geritz et al., 1998). This means that when locally close to this point, the population will converge to this point and will remain at this point if it is ES (if it is not ES, the population can be invaded by mutants that are closer to the point). These conditions can be computed mathematically by checking if certain second-order conditions of the fitness equation are satisfied (we compute these in later chapters).

There are four combinations of ES and CS properties which each give different types of singular points. Firstly, a singular point that is ES and CS is a continuously stable strategy (CSS), also known as an attractor, so the population will evolve towards this point and remain at it once it is reached

Box 1.1: Fitness equation

To obtain the fitness equation for a mutant population trying to invade a resident equilibrium, I begin by considering a resident population which is at equilibrium and look at the possibility of invasion by a rare mutant. I use the stability of this resident-mutant equilibrium, which depends on the eigenvalues from the mutant part of the resident-mutant system's Jacobian matrix, to calculate the fitness for the mutant population (Metz et al., 1996, Geritz et al., 1998). Given that the resident is at a stable equilibrium and the mutant is rare, the fitness only depends on this Jacobian matrix. For the Type I Holling response, these eigenvalues are real and for the Type II and III Holling responses, complex eigenvalues can arise leading to cyclical population dynamics (for which numerical simulations are used, explained in Box 2.6). The following analysis holds true for the adaptive dynamics approach using real eigenvalues (additionally, for eigenvalues which do not result in limit cycles, this is also relevant as we can just focus on the real part to determine stability of the resident to invasion by a mutant). For the model in Chapters 2 and 3, this matrix is given by:

$$\begin{pmatrix} a - qH - b - \beta I - cP & af - qfH + \gamma \\ \beta I & -\Gamma - c\phi P \end{pmatrix}$$

For the model in Chapter 5, this matrix is given by:

$$\begin{pmatrix} -(b+\alpha) - c\phi(P_S + P_I) & \frac{\beta\lambda S}{\mu} \\ c\phi P_S & -d - \alpha_P \end{pmatrix}$$

These matrices can be represented more simply by:

$$J = \begin{pmatrix} A & B \\ C & D \end{pmatrix}$$

where $B > 0$ ($a > qH$ for the first matrix), $C > 0$ and $D < 0$ for my parameter requirements. In the first matrix the sign of A is unknown and in the second matrix $A < 0$. The fitness equation is given by the dominant eigenvalue of the matrix J which is computed below:

$$\begin{vmatrix} A - \lambda & B \\ C & D - \lambda \end{vmatrix}$$

$$= (A - \lambda)(D - \lambda) - BC = \lambda^2 - (A + D)\lambda + (AD - BC) = 0$$

$$\lambda_\pm = \frac{A + D \pm \sqrt{(A + D)^2 - 4(AD - BC)}}{2} = \frac{A + D \pm \sqrt{(A - D)^2 + 4BC}}{2}$$

where λ_- is the eigenvalue using the subtraction option and λ_+ is the eigenvalue using the addition option.

If $A < 0$ then $\lambda_- < 0$ and if $A \geq 0$ then again $\lambda_- < 0$ because $|A + D| \leq |A - D|$. Therefore, $\lambda_- < 0$. Clearly λ_+ is the fitness term as it is the dominant real eigenvalue ($\lambda_+ > \lambda_-$). The terms s or r are used to represent the fitness equations for the host and parasite populations, respectively, so $s, r = \lambda_+$.

The determinant of $J - \lambda I$ is given by $(\lambda_- - \lambda)(\lambda_+ - \lambda)$ and setting $\lambda = 0$, we can see that the determinant of J is simply given by the product of the two eigenvalues, $\lambda_-\lambda_+$. Then considering the following two cases:

- The mutant invades if $s, r = \lambda_+ > 0$, i.e. determinant $\lambda_-\lambda_+ < 0$
- The mutant cannot invade if $s, r = \lambda_+ < 0$, i.e. determinant $\lambda_-\lambda_+ > 0$

Therefore, taking the negative determinant is sign equivalent to the fitness equation computed using the dominant eigenvalue (proved similarly in Appendix A in Hoyle et al., 2012). Hence I use this equation as a fitness proxy to determine the fitness in my analysis.

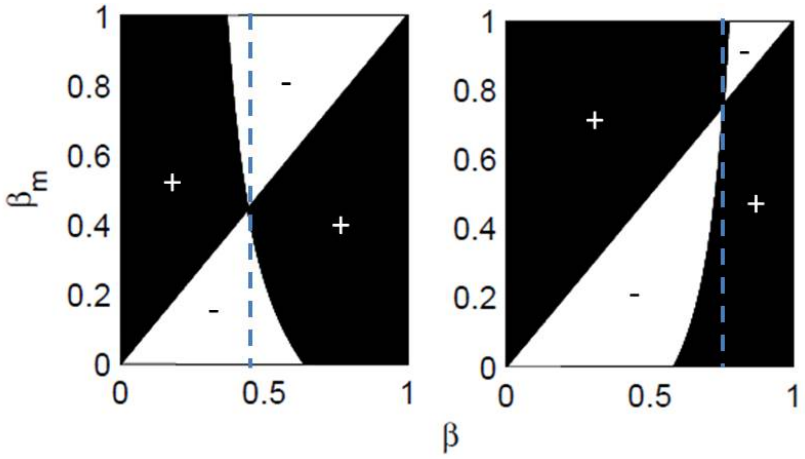


Figure 1.1: PIPs showing CSS and branching points, respectively. The mutant has positive fitness in the shaded region (+) and negative fitness in the unshaded region (−). A dashed vertical line through an unshaded region means the point is ES and a dashed vertical line through a shaded region means the point is not ES. The shaded regions above the diagonal to the left and below the diagonal to the right mean that the points are CS.

giving us the final outcome of evolution. Secondly, a singular point that is CS and not ES is a branching point so the population will tend to move towards this point but once the point is reached, the population will branch into coexisting populations with different traits producing more diversity in the system. Thirdly, a singular point that is ES and not CS is a Garden of Eden point so a population will not converge to this point but will remain at this point if it starts there. Lastly, a singular point that is neither CS nor ES is an evolutionary repellor so the population will move away from this point. Therefore, using this information allows us to determine how evolution of the population will occur.

1.3 Trade-offs

Within the models I study, I include trade-offs in which an increase in investment in one trait leads to a constraint in another. This adds more complexity to the host-parasite systems but makes the models more applicable to natural populations. There is clear evidence that trade-offs must exist (Stearns, 1989) and as mentioned by Boots and Bowers (2004), there is a cost in evolving resistance for the host. Clearly, if there were no costs to immunity through increased defences, selection would fix all species at maximum resistance, therefore, the occurrence of variation in nature suggests that there are costs. There is also experimental evidence showing the existence of various trade-offs. There is evidence that building up defences against an infection is costly to the life-history traits of hosts, such as their birth rate (Stearns, 1989, Boots and Begon, 1993). There is also evidence showing that as hosts increase defences against one enemy, they constrain their defences to another enemy (Rigby and Jokela, 2000, Stinchcombe and Rausher, 2001, Nuismer and Thompson, 2006, Craig et al., 2007, Edeline et al., 2008, Gomez et al., 2009, Siepielski and Benkman, 2009, Friman and Buckling, 2012). For example, Rigby and Jokela (2000) found that as *Lymnaea stagnalis* freshwater snails increased investment in predator avoidance behaviour, they lowered their immune defences against potential pathogens. There is also evidence of trade-offs occurring in parasites which are able to evolve manipulation of an intermediate host in order to reach their final host as it can be costly to their life history traits (Vizoso and Ebert, 2005, Frost et al., 2008, Franceschi et al., 2010, Cressler et al., 2014). For example, Franceschi et al. (2010) found that parasites that develop rapidly do not induce behavioural changes in their host, whereas, parasites that develop slowly are able to manipulate the behaviour of the host, suggesting that investment in manipulation leaves less energy for parasite growth. Hence, due to this vast empirical evidence of trade-offs, it is important include them in host-parasite models.

The costs of developing defence are determined by the shape of the trade-

off curve (see Figure 1.2; Hoyle et al., 2008). Simple linear trade-offs are fairly unrealistic as it is unlikely that such perfectly linear trade-offs would occur due to the complexities that arise in nature, therefore, non-linear trade-offs are typically considered. Decreasingly costly trade-offs are characterised by a convex curve. Intuitively, we would expect these types of trade-offs to be unlikely in nature as costs cannot continue to decline for an infinite amount of time. Increasingly costly trade-offs are characterised by a concave curve which seem more likely as given the costs, there is typically evolution to an intermediate value as investing more may be too costly and less efficient. Using a sigmoidal trade-off, where the evolutionary trait reaches maximum efficiency then becomes increasingly costly, may seem more realistic but the outcome produced is the same as that of a convex curve (Boots and Haraguchi, 1999). In our models, it is most likely that investment in a strategy comes at an increasingly costly price so we include concave trade-off curves. We later consider varying the trade-off curvature within our models.

Given the wide variety of trade-offs which can occur within host-parasite systems, there are multiple ways to consider these cases and in each of our models we consider a specific trade-off. In Chapter 2, we consider a trade-off in the host, between their birth rate and defence levels (Figure 2.2), such that when the hosts increase their defences against infection by the parasite (thereby lowering the transmission rate), there is a cost as they lower their birth rate. In Chapter 3, when the hosts increase their defences against one enemy (the parasite or predator), we assume that they constrain their defence against the other, giving a trade-off in defence against the two enemies (Figure 3.1). In Chapter 4, we aim to determine the trade-off occurring in an experiment with *Plodia interpunctella* (Indian meal moth) and two enemies (virus and bacteria), making predictions of what type of trade-off, if any, may exist (Section 4.1.1). Lastly, in Chapter 5, we consider the case where the predator is no longer immune to infection as it acts as the final host for the parasite, with the prey now acting as an intermediate

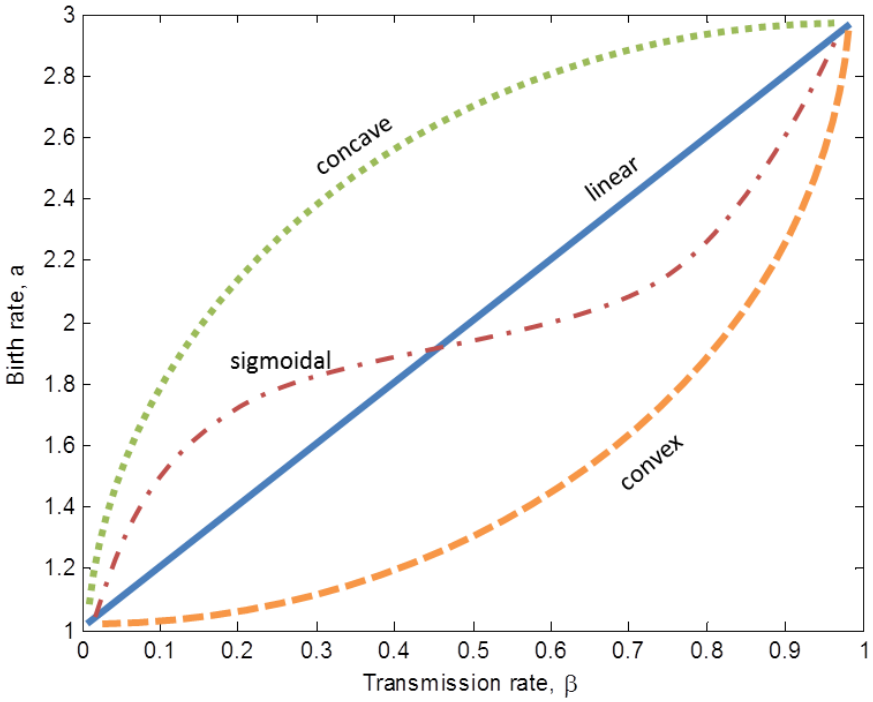


Figure 1.2: Illustration of various types of trade-off curves using a trade-off between host birth rate (a) and disease transmission rate (β).

host. Here, we include a trade-off in the parasite, between manipulation and spore production, such that when the parasite increases manipulation of their intermediate host, making them more conspicuous to the predator, they lower their spore production (Figure 5.2).

1.4 Overview

In the past, theoretical models on the evolution of host defences against disease have been widely studied but the inclusion of predators has often been overlooked. The first part of my work in Chapter 2 considers the evolution of host defence against infection in the presence of a predator population. I include a trade-off between host resistance and birth rate, such that an increase in host resistance lowers their birth rate. One key insight of this work is that the inclusion of the predator population impacts the evolution of the hosts defence mechanisms. The presence of the predator population also results in increased host diversity. Therefore, this work highlights the importance of the inclusion of the predator population when studying host-parasite systems as it has an impact on evolution. Parts of Chapter 2 are published in the *Journal of Theoretical Biology* (see Toor and Best, 2015).

Due to limited resources, when increasing defences against one enemy, hosts may constrain their defences against another. In Chapter 3, I theoretically examine the evolutionary behaviour of a host population which must allocate defences between two enemy populations, parasites and predators. Here, I include a trade-off such that an increase in defence against one enemy constrains defence against the other. Since this behaviour has been seen in various experimental studies, I determine theoretically how the host population decides which enemy to defend themselves against and when they are more likely to defend themselves against a particular enemy. The surrounding enemy community composition and the population growth rates are key when considering the complexity in this system. Additionally, greater host

diversity is likely when the host faces a simultaneous balanced risk of infection and predation. Parts of Chapter 3 are published in the *American Naturalist* (see Toor and Best, 2016).

In Chapter 4, I discuss the methods and results of experimental work carried out in Professor Mike Boots lab at the University of Exeter, Penryn campus, on *Plodia interpunctella* (Indian meal moth) to look at the evolution of resistance against virus and bacteria. I aim to determine whether larvae which have built up resistance to the virus are more or less resistant to bacteria (or if there is no effect). By analysing the data, the results should reveal whether there is a trade-off between defence or a generalised defence mechanism acting against these two enemies. Although the data proves non-significant, I highlight the importance of such experiments as the integration of theoretical host-parasite models with experimental studies is vital.

The parasite population may evolve manipulation when the predator is no longer immune to infection. Here, the prey acts as an intermediate host which is manipulated by the parasite increasing its likelihood of reaching and infecting the final host, the predator. Experimental studies have revealed that parasites may evolve manipulation of their intermediate host in order to increase their chances of transmission to the final host, however, it is not clear when we should expect parasites to evolve such a manipulation strategy if it is costly to their life history traits. In Chapter 5, I theoretically study an intermediate host-parasite model with a predator population, whilst including a trade-off in the parasite, between manipulation and spore production. I determine the conditions under which parasites benefit from investing in such costly manipulation finding that the population dynamics are once again very important.

We end in Chapter 6, with a discussion of the main results and potential ideas for future work in this area. Overall, my work highlights the importance of the inclusion of the predator population when studying host-parasite systems as I have shown that it has an impact on the evolution of the host and

parasite populations. By considering these complexities which need to be accounted for when modelling such systems, my work has provided us with many new insights in this growing field and further studies will continue to improve our understanding of infectious diseases.

Chapter 2

The evolution of host resistance to disease in the presence of predators

2.1 Introduction

Many mathematical models have studied the evolution of host defences to parasitism, emphasising the importance of ecological feedbacks to selection (van Baalen, 1998, Boots and Bowers, 1999, Boots and Haraguchi, 1999, Roy and Kirchner, 2000, Gandon et al., 2002, Restif and Koella, 2003, Boots, 2004, Restif and Koella, 2004, Miller et al., 2005, Bonds, 2006, Miller et al., 2007, Boots et al., 2009). Whilst these studies have given us considerable insight into the evolutionary ecology of host defences to disease, most of these models do not take community interactions into consideration. In nature, host-parasite populations are often imbedded in a wider community and

Parts of this chapter are in the paper (see Toor and Best, 2015) published in the *Journal of Theoretical Biology*.

are therefore affected by other factors in the environment, such as resource availability, interspecific competition and predation. To fully understand the evolutionary dynamics of host-parasite interactions in natural communities, it is vital that we explore the impact of these wider interactions.

In recent years, theoretical studies have begun to emerge exploring the impacts of predation on parasite evolution. Morozov and Adamson (2011) showed that the presence of a predator can lead to the evolution of highly virulent parasites which may ultimately lead to predator extinction. In another study, Morozov and Best (2012) showed that predation allows for parasite diversity through evolutionary branching rather than classic R_0 maximisation (Bremermann and Pickering, 1983, Bremermann and Thieme, 1989), while Kisdi et al. (2013) found that predation can lead to evolutionary cycles of pathogen virulence and predator density. In the only previous study on the impacts of predation to host evolution to date, Hoyle et al. (2012) examined the effects of predation on host evolution of resistance towards parasite and predator exclusion, as well as showing that there are greater branching possibilities in the host. Despite this work, we still have limited general understanding of the effects of predation on host investment in defences to parasitism.

Clearly, the main effect of predation on a host population is the creation of additional mortality. There is some empirical evidence showing that predators are likely to selectively predate on infected prey since they may be easier to catch. An example of such selective predation can be seen in red grouse populations, as birds with higher parasite burdens are more prone to predation because they emit more scent to predators when heavily infected (Hudson et al., 1992). Similar findings have been shown for snowshoe hare populations as parasites also make them more vulnerable to predators (Murray et al., 1997).

Previous research has also shown that the presence of a predator affects the evolutionary outcomes of experimental systems. Bacteria-phage exper-

iments have been conducted by Friman and Buckling (2012) which showed that the inclusion of a protist (predator) lead to bacterial (host) diversification and increased bacterial resistance against protists. When the bacteria were placed in an environment with phage (virus) and protists, building defence against one enemy constrained their defence against the other. Due to this, the bacteria lost almost all resistance against phage whilst coevolving with the protist (Friman and Buckling, 2012).

In this chapter, I focus on the impact of an additional predator on a host-parasite system. I assume that hosts can evolve increased resistance through lowered transmission, but that there is a trade-off to their birth rate. Working within the framework of adaptive dynamics (Geritz et al., 1998), I use pairwise invasibility plots (PIPs) and run numerical simulations to look at the evolutionary behaviour of the system, specifically considering how stable investment in resistance varies with predation and how predation impacts the potential for evolutionary branching.

2.2 Model

A host-parasite model with a predator was used, as used by Hoyle et al. (2012) but with the inclusion of an infected fecundity term (f) and varying Holling Type responses (ρ_S, ρ_I). The model is given by the following differential equations:

$$\frac{dS}{dt} = a(S + fI) - qH(S + fI) - bS - \beta SI + \gamma I - \rho_S(S)P \tag{2.1}$$

$$\frac{dI}{dt} = \beta SI - (\alpha + b + \gamma)I - \rho_I(I)P \tag{2.2}$$

$$\frac{dP}{dt} = \theta P(\rho_S(S) + \rho_I(I)) - dP \tag{2.3}$$

where S, I and P are the densities of susceptible prey, infected prey and predators, respectively with $H = S + I$. A schematic diagram of the model is

Table 2.1: Parameter definitions and values used

Parameter	Definition	Default Value
β	Transmission rate	Varies
a	Prey birth rate	Varies
α	Parasite-induced death rate/virulence	0.2
ϕ	Increase/decrease in the predation rate suffered by infected individuals	3
θ	Conversion of predation into births of new predators	1
γ	Recovery rate	0.2
q	Rate of density-dependent competition, acting on births	0.5
b	Natural prey death rate	0.2
d	Predator death rate	0.3
f	Infected fecundity (proportion of infected individuals able to reproduce)	1
Γ	1/Infectious period, $\alpha + b + \gamma$	0.6
c	Predation rate	Varies
ρ_S, ρ_I	Holling Type I, II or III response	Varies

shown in Figure 2.1 with the parameters defined in Table 2.1 (the parameter region was selected by producing numerous population density plots to ensure that a region was chosen where the susceptible and infected hosts coexist with the predator population). In this model, once a host is infected it moves from the susceptible class to the infected class as transmission is assumed to occur through contact of a susceptible individual with an infected individual at transmission rate β. If the infected host recovers, it moves back into the susceptible class at recovery rate γ as it can be re-infected by the parasite (susceptible-infected-susceptible [SIS] model; Anderson and May, 1981). I assume that the predator is immune to infection in this system so an infected host can be consumed without the predator becoming infected. This becomes an SI model as the infected fecundity (f) and recovery (γ) terms approach zero.

I begin by using a Holling Type I response (linear response) which assumes that the number of prey (hosts) consumed by predators increases linearly with prey density, hence this response does not limit the predation rate of the predator (Real, 1979, Fujii et al., 1986). Holling Type II and III responses were also studied (see Section 2.3.5). For the Type I model, $\rho_S(S) = cS$ and $\rho_I(I) = c\phi I$, where $\phi > 1$ corresponds to selective predation on infected

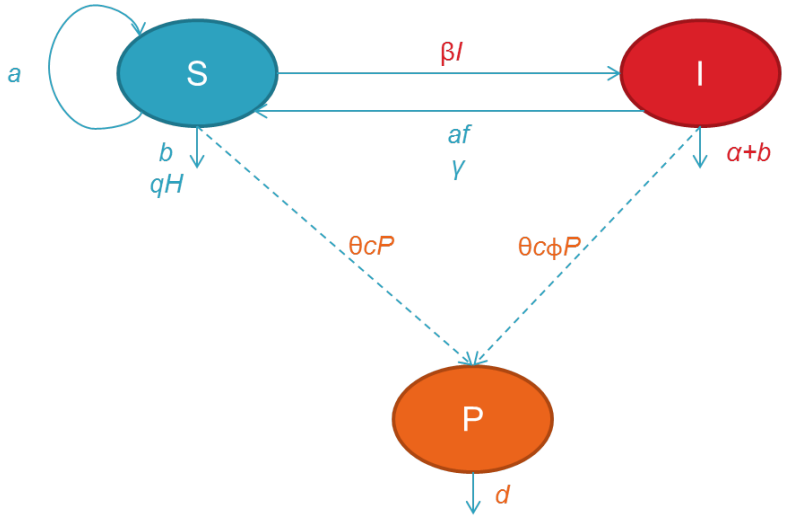


Figure 2.1: Flowchart diagram of model, where S, I and P are the densities of susceptible prey, infected prey and predators, respectively (parameters are defined in Table 2.1). The dashed lines represent predation processes.

prey. I focus on predators that selectively predate on infected hosts (i.e. $\phi > 1$ in our model), although I also consider varying this level of selective predation. This Type I model leads to stable dynamics where the susceptible and infected hosts coexist with the predator population or the infected host and/or predator population are excluded (see Box 2.1).

I explore the evolutionary dynamics of host resistance whilst working within the framework of adaptive dynamics (Geritz et al., 1998), assuming that we start with a resident strain at equilibrium and then determine whether small, rare, nearby mutant strains can invade the population and replace the current resident. For the Type II and III responses, there is some cyclic behaviour emerging, which becomes more prominent when there is no recovery and the infected hosts are sterile. For the Type I model, the fitness equation for a mutant host invading a resident equilibrium is obtained by considering the invasion of a rare mutant into an environment where a resident strain is at equilibrium. By considering the stability of this resident-

Box 2.1: Population dynamics

Throughout my results for Chapters 2 and 3, I focus on the region where all of the populations (S, I and P) coexist. There are regions where the predator is excluded, such that S and I coexist and regions where the parasite is excluded, such that S and P coexist (regions shown in Figure 2.9). These parasite and predator exclusion boundaries can be calculated using R_0 (see Box 2.3).

Additionally, I focus on using a Type I Holling response where the fitness can be calculated using the method shown in Box 2.2. The parameter values selected ensure that the dynamics remain stable with real eigenvalues.

For the Type II and Type III Holling responses, imaginary eigenvalues can arise and it becomes intractable to obtain the explicit equilibrium population densities so the method in Box 2.2 can no longer be used. Hence, numerical simulations are used, following the method in Box 2.6 to determine the evolutionary outcome. In this case, limit cycles arise leading to cyclic dynamics in the population densities.

mutant equilibrium, we can calculate the sign-equivalent proxy equation for the mutant's invasion fitness to be:

$$s(\hat{a}, \hat{\beta}; a, \beta) = (\hat{a} - qH - b - \hat{\beta}I - cP)(\Gamma + c\phi P) + \hat{\beta}I(\hat{a}f - qfH + \gamma) \quad (2.4)$$

Here I use $\hat{a}$ and $\hat{\beta}$ to represent the mutant strain traits (see Box 2.2 for the full derivation of the fitness equation). If the mutant has positive fitness ($s > 0$), it invades becoming the new resident and we repeat the process again to see if it is then invaded. If the mutant has negative fitness ($s < 0$), it cannot invade so the current resident remains. If $s = 0$, the mutant population has zero fitness. We continue this process until the final outcome of evolution is determined, i.e. the trait is no longer evolving as it stays fixed at a point or has diverged into two coexisting populations.

The mutant strains vary along the trade-off curve which I assume is between host resistance, through a reduced transmission rate, and birth rate

such that higher resistance (reducing the transmission rate) corresponds to a lower birth rate and vice versa (shown in Figure 2.2; concave trade-off with costs of resistance become increasingly costly to births). The trade-off curve, $a(\beta)$, is given by:

$$a(\beta) = a(\beta^*) - \frac{a'(\beta^*)^2}{a''(\beta^*)}(1 - e^{\frac{a''(\beta^*)(\beta-1)}{a'(\beta^*)}}) \tag{2.5}$$

Here I use primes to denote derivatives, for example, $a'(\beta^*) = \frac{\partial a}{\partial \beta}\big|_{\beta=\beta^*}$ (same format of trade-off equation as used by Hoyle et al. (2012)). This trade-off equation allows us to fix the singular point, (β^*, a^*) and select the slope, $a'(\beta^*)$, and curvature, $a''(\beta^*)$, of the trade-off curve at that point, which will have an important impact on the evolutionary outcome of this model. In particular, the slope determines that the singular point is an evolutionary singularity (see equation 2.6) and the curvature determines the behaviour at that point. For our results, I set $(\beta^*, a^*) = (1, 2)$, $a'(\beta^*) = 0.078$ and $a''(\beta^*) = -0.15$. Evolutionary singular points occur when the gradient of the fitness equation is zero:

$$\frac{\partial s}{\partial \hat{\beta}} \,|_{\hat{\beta}=\beta} = (a'(\beta) - I)(\Gamma + c\phi P) + I(af - qfH + \gamma) + f\beta I a'(\beta) = 0 \tag{2.6}$$

I start by looking for continuously stable strategy (CSS) points, which are evolutionarily stable (ES, meaning the population will stay at this point once reached as no nearby mutants can invade) and convergence stable (CS, meaning the population will converge to this point when close to it) (Geritz et al., 1998). I then move on to look at the possibility of two strains coexisting through branching.

Box 2.2: Fitness equation

To obtain the fitness equation for a mutant population trying to invade a resident equilibrium, I begin by considering a resident population which is at equilibrium and look at the possibility of invasion by a rare mutant. I use the stability of this resident-mutant equilibrium to calculate the fitness for the mutant population.

The following are the equations for the mutant population:

$$\frac{d\hat{S}}{dt} = \hat{a}(\hat{S} + f\hat{I}) - q(\hat{S} + f\hat{I})H - b\hat{S} - \hat{\beta}\hat{S}I + \gamma\hat{I} - c\hat{S}P \tag{2.7}$$

$$\frac{d\hat{I}}{dt} = \hat{\beta}\hat{S}I - (\alpha + b + \gamma)\hat{I} - c\phi\hat{I}P \tag{2.8}$$

where $H = S + I$. The stability of the resident-only equilibrium is found to depend on the eigenvalues from the mutant part of the resident-mutant system's Jacobian matrix, given by:

$$\begin{pmatrix} \hat{a} - qH - b - \hat{\beta}I - cP & \hat{a}f - qfH + \gamma \\ \hat{\beta}I & -\Gamma - c\phi P \end{pmatrix}$$

where $\Gamma = \alpha + b + \gamma$.

The fitness equation is given by the dominant eigenvalue of this matrix. Alternatively, we take the negative determinant of the above matrix, which gives:

$$s(\hat{a}, \hat{\beta}; a, \beta) = (\hat{a} - qH - b - \hat{\beta}I - cP)(\Gamma + c\phi P) + \hat{\beta}I(\hat{a}f - qfH + \gamma) \tag{2.9}$$

This equation is sign equivalent to the fitness equation computed using the dominant eigenvalue of this Jacobian matrix (see Box 1.1 in Chapter 1 for proof). Hence I use this equation as a fitness proxy to determine the fitness in my analysis.

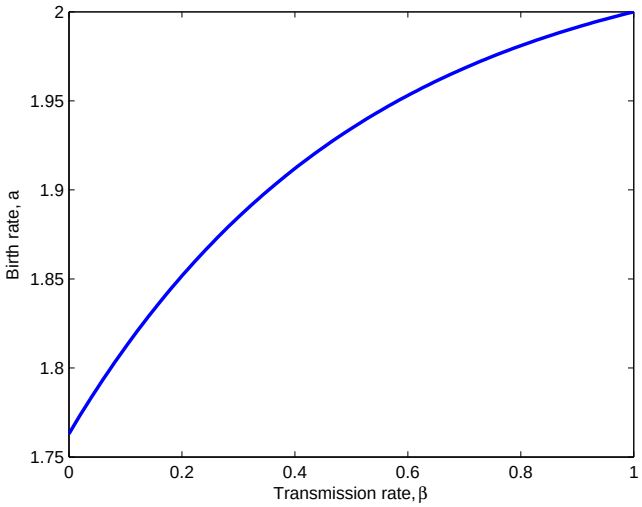


Figure 2.2: Trade-off curve between host birth rate (a) and transmission rate (β) where $(\beta^*, a^*) = (1, 2)$, $a'(\beta^*) = 0.078$ and $a''(\beta^*) = -0.15$.

2.3 Results

I focus on the impact of predation on the evolution of host resistance. Therefore, I analyse how CSS investment in defence (which lowers the CSS transmission rate, β^*) varies with increasing predation rate, c, and determine how this response changes as other parameters are varied. I focus on the region where the susceptible and infected hosts coexist with the predator population, although there are regions where the parasite, predator or both are excluded (for the parameter region of interest, a single CSS or branching point exists; see Boxes 2.3 and 2.4 for the exclusion thresholds). There are two main factors that come into play, firstly the chance of becoming infected which is dependent upon the infected population density; I will refer to this as the *risk of infection* and secondly, the chance of being preyed upon after infection which is dependent upon the predator population density; I will refer to as the *cost of infection*. The general shape we see is a 'U-shaped' curve, as can be seen in Figure 2.3, here, a higher transmission rate corre-

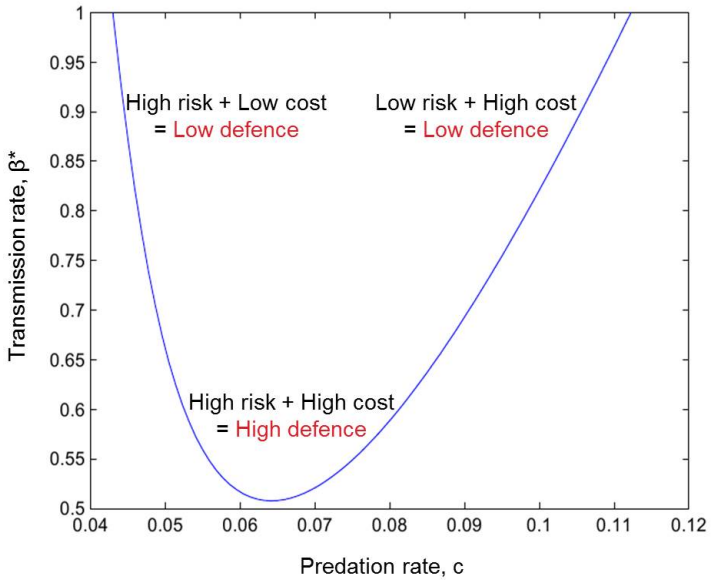


Figure 2.3: Host investment in defence as the predation rate (c) varies, where the cost of infection relates to the infected population and the risk of infection relates to the predator population, using parameter values given in Table 2.1. Low β^* corresponds to high host defence and vice versa.

sponds to lower host defence. At low c, there is a high risk of infection due to a large infected population but there is a low cost of infection since there is a small predator population and so additional mortality is not too high. As c increases, the exposure risk falls slightly but the cost of infection, due to predation, rises so there is greater selection for defence (β^* declines). Then at high c, although there is a high cost of infection (due to a large predator population), the risk of exposure has fallen sufficiently that selection for defence decreases (β^* increases) as most of the infected hosts have been removed through predation. Therefore, maximum defence (low β^*) occurs at intermediate c values, for which the susceptible and infected hosts coexist with the predator, as this is where the hosts face the greatest combined risk and cost of infection.

Box 2.3: Exclusion thresholds

The inclusion of the predator in this system gives us two exclusion thresholds leading to cases where the parasite, predator or both may be excluded from the population. The results agree with those found by Hoyle et al. (2012), as when the predation rate is low, the predator is at risk of being excluded and when the predation rate is high, the parasite faces this risk of exclusion. The reproductive rate, R_0, shows whether the population (of infected hosts or predators) is increasing or decreasing. For a population to increase, the reproductive rate must be greater than one, i.e. $R_0 > 1$. If the reproductive rate is less than one, i.e. $R_0 < 1$, the population will decrease. Hence, the threshold is given when $R_0 = 1$. The exclusion thresholds (determined by R_0) occur at low c where the predator population is excluded and at high c where the infected hosts are excluded. The parasite-exclusion threshold, R_{0I}, is the number of secondary infections caused by a primary infection in a wholly susceptible population. This threshold can be found by checking when $\frac{dI}{dt} > 0$ at $I = 0$ using equation 2.2.

$$\frac{dI}{dt} = \beta SI - (\alpha + b + \gamma)I - c\phi IP > 0$$

$$\beta SI > \Gamma I + c\phi IP$$

$$\frac{\beta S}{\Gamma + c\phi P} > 1$$

Therefore, the parasite exclusion threshold is given by the following reproductive rate:

$$R_{0I} = \frac{\beta S^*_{SP}}{\Gamma + c\phi P^*_{SP}} = 1 \tag{2.10}$$

where S^*_{SP} and P^*_{SP} are the equilibrium densities of the susceptible prey and the predators in the absence of the parasite ($I^*_{SP} = 0$). The predator-exclusion threshold, R_{0P}, is the number of offspring a single predator would produce during its lifetime in a wholly prey environment. This threshold can be found similarly by checking when $\frac{dP}{dt} > 0$ at $P = 0$ using equation 2.3.

$$\frac{dP}{dt} = \theta cP(S + \phi I) - dP > 0$$

$$(S + \phi I) > \frac{d}{\theta c}$$

$$\frac{\theta c}{d}(S + \phi I) > 1$$

Therefore, the predator-exclusion threshold is given by the following reproductive rate:

$$R_{0P} = \frac{\theta c}{d}(S^*_{SI} + \phi I^*_{SI}) = 1 \tag{2.11}$$

where S^*_{SI} and I^*_{SI} are the equilibrium densities of the susceptible and infected prey in the absence of the predator ($P^*_{SI} = 0$) (reproductive rates also computed in Hoyle et al., 2012).

Box 2.4: Example using R_0

We can calculate the R_0 values to determine the parasite-exclusion threshold and the predator-exclusion threshold. For example, the thresholds are computed below for $\alpha = 0.6$. For the parasite-exclusion threshold, I will check when $R_{0I} < 1$ using equation 2.10. By using the relevant equilibrium values and parameter values defined in Table 2.1 (and setting $\alpha = 0.6$), the following calculations can be made:

$$S_{SP} = \frac{d}{c\theta} = \frac{0.3}{c}$$

$$P_{SP} = \frac{-dq + ac\theta - bc\theta}{c^2\theta} = \frac{1.8c - 0.15}{c^2}$$

$$R_{0I} = \frac{\beta S_{SP}}{\alpha + b + \gamma + c\phi P_{SP}} = \frac{0.3}{6.4c - 0.45} < 1 \rightarrow c > 0.1171875$$

Therefore, for approximately $c > 0.117$, the parasite is excluded.

For the predator-exclusion threshold, I will check when $R_{0P} < 1$ using equation 2.11. Similarly to the previous calculation, the following calculations can be made:

$$S_{SI} = \frac{\alpha + b + \gamma}{\beta} = 1$$

$$I_{SI} = \frac{-(\beta\alpha - a\beta + \beta b + 2\alpha q + 2bq + 2\gamma q}{2\beta q}$$

$$\frac{-\beta\sqrt{\frac{\beta a^2+\beta\alpha^2+\beta b^2+4\alpha^2 q-2a\beta\alpha-2a\beta b+2\alpha\beta b+4\alpha bq+4\alpha\gamma q}{\beta}})}{2\beta q}$$

$$= 1.8248$$

$$R_{0P} = \frac{\theta c}{d}(S_{SI} + \phi I_{SI}) = \frac{6.4744c}{0.3} < 1 \rightarrow c < 0.04633634$$

Therefore, for approximately $c < 0.046$, the predator is excluded. This can be seen in Figure 2.6b.

2.3.1 Pairwise Invasibility Plots (PIPs)

To verify the results, pairwise invasibility plots (PIPs) were produced whilst varying the predation rate, c. The PIPs are produced by computing the fitness equation (equation 2.4) and checking when it is positive or negative. Along the boundary curves, $s = 0$. For low c, the predators are excluded, whereas, for high c, the infected hosts are excluded. For intermediate c, all three populations (S, I, P) coexist and are greater than zero. The PIPs shown in Figure 2.4 confirm that when c is small, the hosts have low defences (high transmission rate) as there is a low cost of being infected. Then as c increases, the predator population increases so the hosts build up their defences (lower transmission rate) to avoid becoming infected. Once large values of c are reached, there are not many infected hosts remaining so the hosts lower their defences (higher transmission rate).

2.3.2 Predation rate suffered by infected individuals and virulence

As selective predation (ϕ) or virulence (α) increase, the cost of infection increases, due to higher mortality through predation or infection once infected with the disease. This leads to greater levels of defence (lowering transmission rates) for higher values of ϕ or α, with maximum defence reached at lower predation rates as shown in Figures 2.5a and 2.5b. As c continues to increase, the infected density is lower for higher ϕ or α (as shown in Figures 2.6a and 2.6b) so there is a decrease in the prevalence of infection, allowing the hosts to lower their defence levels and focus on increasing their birth rate.

Figure 2.5a shows that the general 'U-shaped' curve pattern remains the same regardless of whether the predators prefer to consume the infected prey (corresponding to $\phi > 1$), the susceptible prey ($\phi < 1$) or both equally ($\phi = 1$). The hosts maximise their defence at intermediate c both for $\phi \geq 1$

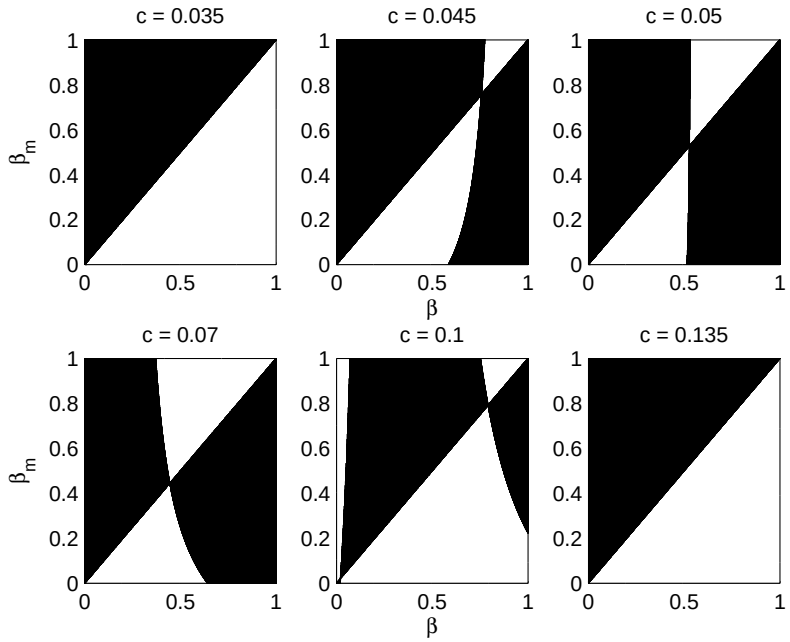


Figure 2.4: PIPs for varying predation rate (c) values, where $a''(\beta^*) = -0.1$. For $c = 0.035$, there are no predators in the population. For $c = 0.045$ and 0.05, there is a branching point. For $c = 0.07$ and $c = 0.1$, there is a CSS transmission rate (β^*). For $c = 0.135$, there are no infected prey remaining in the population so β^* does not exist.

and for $\phi < 1$ as this is where the risk and cost of infection is highest in either case. As ϕ increases, the curve tends to shift downwards as the hosts increase their resistance more drastically due to the rising cost of infection (similar for α as shown in Figure 2.5b).

2.3.3 Other parameters

The conversion of predation into births of new predators (θ) (and also recovery rate, γ, for very low c) behaves similarly to the predation rate suffered by infected individuals (ϕ) and virulence (α) terms. As θ increases, the hosts maximise their defence levels at intermediate c but lower values (the curve is shifting to the left as θ increases as shown in Figure 2.5c) because the predator population is growing faster leading to a high cost of infection earlier as shown in Figure 2.6a. Overall, we find that an increase in these parameters reduces the size of the infected population, thereby lowering the risk of infection.

For small predator death rate (d) values (refer to $d = 0.2$ in Figures 2.5d and 2.6c), at low c, there are already many predators present in the population so the hosts focus on increasing their defence mechanisms, reaching maximum defence for lower c values where all three populations coexist. The infected population then decreases due to the high levels of predation so the hosts lower their resistance. For large d values (refer to $d = 0.5$ in Figures 2.5d and 2.6c), the hosts once again maximise their defence levels at intermediate c, but higher values (the curve is shifting to the right as d increases) because the predator population takes longer to establish and grow large enough to sufficiently increase the cost of infection.

As the rate of density-dependent competition acting on births (q) or natural prey death rate (b) terms increase, the hosts once again maximise their defence levels at intermediate c, but higher values (the curve is shifting to the right as q or b increase) as the predator population takes longer to sufficiently increase the cost of infection. Also, as there is a lower cost of infection as q

Table 2.2: Summary of effect of parameters on CSS transmission rate (β^*) and population densities

Parameter	At low c: ↑ parameter effect on β^*	At high c: ↑ parameter effect on β^*	Initial population densities: ↑ parameter effect
α	$\downarrow \beta^*$	$\uparrow \beta^*$	$\uparrow S, \downarrow I, \downarrow P$
ϕ	$\downarrow \beta^*$	$\uparrow \beta^*$	$\uparrow S, \downarrow I, \uparrow P$
θ	$\downarrow \beta^*$	$\uparrow \beta^*$	$\uparrow S, \downarrow I, \uparrow P$
γ	$\uparrow \beta^*$ (except for very low c, $\downarrow \beta^*$)	$\uparrow \beta^*$	$\uparrow S, \downarrow I, \downarrow P$
q	$\uparrow \beta^*$	$\downarrow \beta^*$	$\downarrow S, \downarrow I, \downarrow P$
b	$\uparrow \beta^*$	$\downarrow \beta^*$	$\downarrow S, \downarrow I, \downarrow P$
d	$\uparrow \beta^*$	$\downarrow \beta^*$	$\downarrow S, \uparrow I, \downarrow P$

or b increase, the hosts lower their maximum investment in defence (higher transmission rates). This is shown in Figures 2.5e and 2.5f as we see the curves shift upwards and to the right as these parameters increase. Similarly, as the recovery rate (γ) increases, there is a lower cost of infection so the hosts decrease their resistance (the curve shifts upwards as γ increases; see Figure 2.5h). However, at very low c, as γ increases, the risk of infection increases as the initial increase in the number of susceptible prey leads to an increase in the number of infected prey so the hosts increase their defences (lower transmission rates; see Figure 2.5g). As γ continues to increase, there are less infected prey leading to less predators (similar to population dynamics shown in Figure 2.6c). Hence, we find that an increase in these parameters gives the predator population a longer time to establish, thereby lowering the cost of infection. Table 2.2 summarises these results.

2.3.4 Infected fecundity

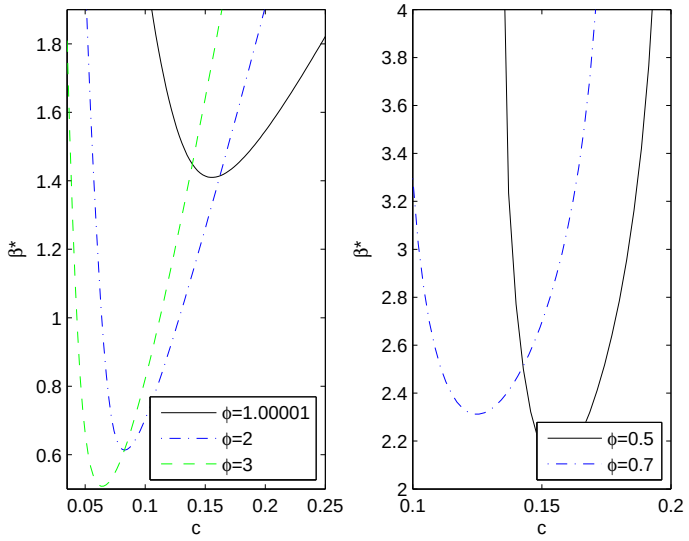
We now look at the effect of varying the infected fecundity (f) on the results. As f approaches zero, there is a smaller proportion of infected individuals which are able to reproduce and when $f = 0$, the infected population is completely sterile. Figure 2.5i shows that as f approaches zero, the initial part of the curve changes as the hosts maintain steady levels of defence until the predators establish in the population. At low c, the predators have

not established in the population so the hosts do not vary their levels of defence, i.e. the β^* value does not vary as shown by the initial flat line in Figure 2.5i. Additionally, the infected prey only reach a small density as f approaches zero so the hosts do not need to increase resistance. Once the predator population establishes and starts removing the infected hosts, the hosts lower their resistance as before because the risk of exposure is further reduced. For f larger than zero, we see that the curve shifts upwards as the hosts lower their levels of defence (higher transmission rates).

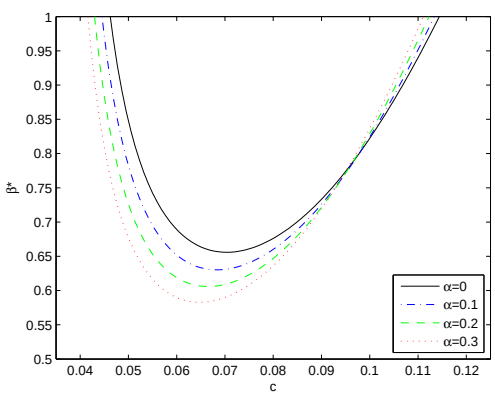
2.3.5 Holling Type II and Type III responses

I also considered a predator using Holling Type II and III responses by modifying ρ_S and ρ_I in the model. Additional parameters are required, the new parameters, h_s and h_i are the half-saturation constants for the susceptible and infected hosts, respectively. In the Type II case (hyperbolic response), the predator has a decelerating intake rate as the number of prey increase because the predator is limited by its capacity to intake prey and by the time it takes to consume them (Real, 1979, Fujii et al., 1986). For the Type II response, $\rho_S(S) = \frac{cS}{S+h_s}$ and $\rho_I(I) = \frac{c\phi I}{I+h_i}$. In the Type III case (sigmoid response), at high levels of prey, saturation occurs and at low levels an accelerating function is caused by learning time or prey switching behaviour (Real, 1979, Fujii et al., 1986). For the Type III response, $\rho_S(S) = \frac{cS^2}{S^2+h_s}$ and $\rho_I(I) = \frac{c\phi I^2}{I^2+h_i}$. Refer to Box 2.5 for the fitness equation and gradient using the Holling Type II and III responses. Figure 2.7 illustrates the different types of Holling responses.

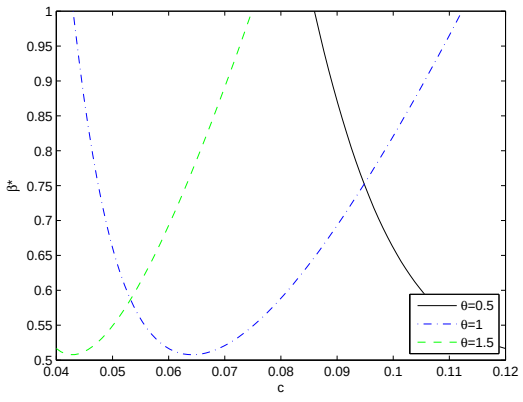
For the Type II and III cases, it becomes intractable to obtain explicit population densities so we rely on the results produced by running numerical simulations (see Box 2.6). We see particularly for the Type III response that the curves from the simulations become less smooth (see Figure 2.8). Numerical simulations of the underlying population dynamics reveal that often these cases result in limit cycles rather than attraction to a stable



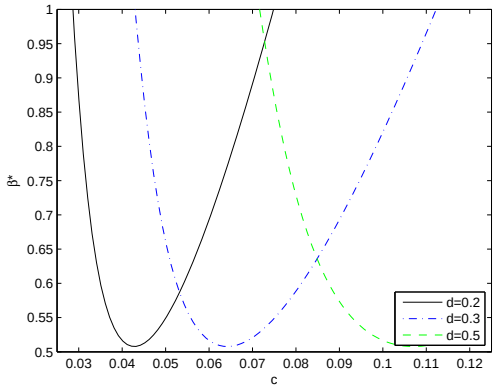
(a) Varying ϕ.



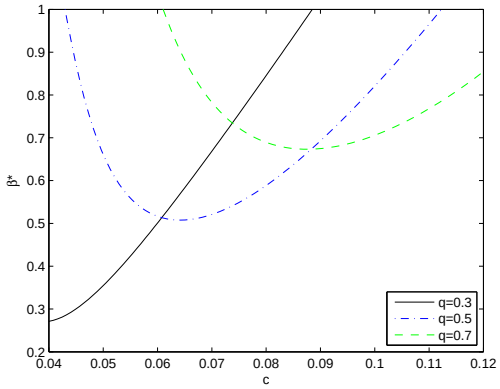
(b) Varying α.



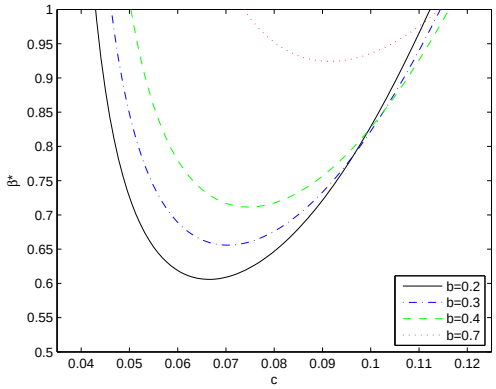
(c) Varying θ.



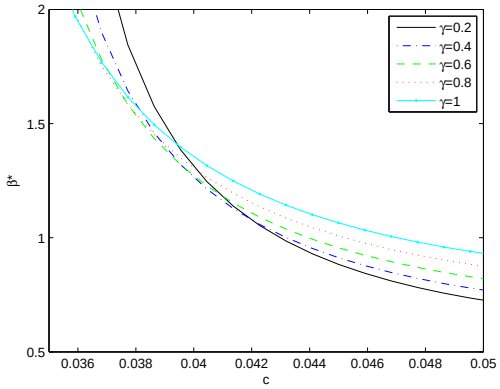
(d) Varying d.



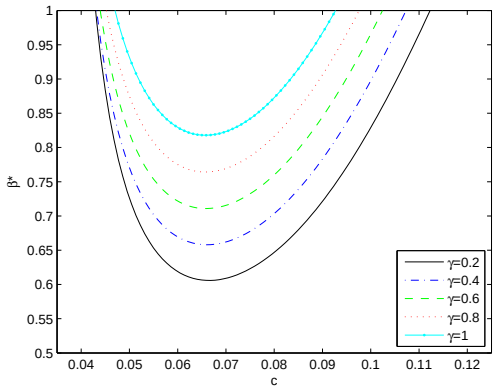
(e) Varying q.



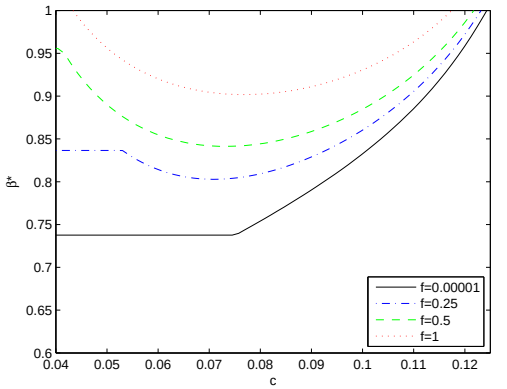
(f) Varying b.



(g) Varying γ for very low c values.

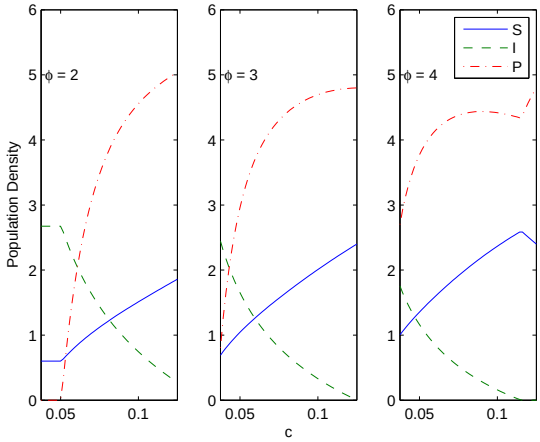


(h) Varying γ.

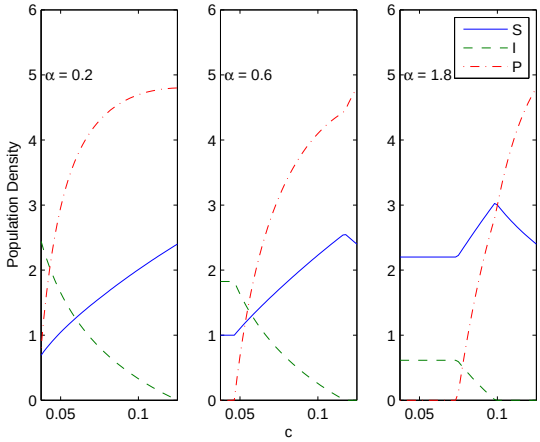


(i) Varying f, where $a''(\beta^*) = -0.8$.

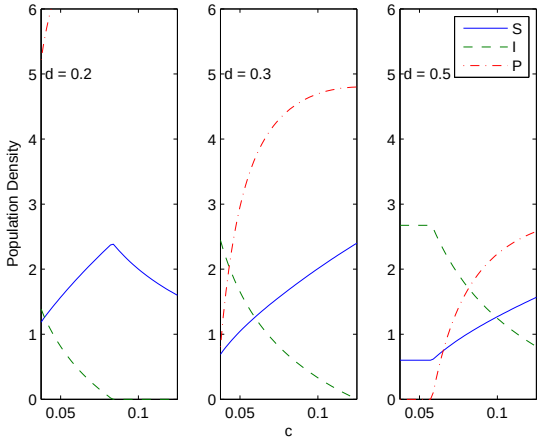
Figure 2.5: CSS transmission rate (β^*), using parameter values given in Table 2.1.



(a) Varying ϕ (similar for θ).



(b) Varying α.



(c) Varying d (similar for q, b and γ).

Figure 2.6: Population densities, using parameter values given in Table 2.1 and $(\beta^*, a^*) = (1, 2)$ at the singular point.

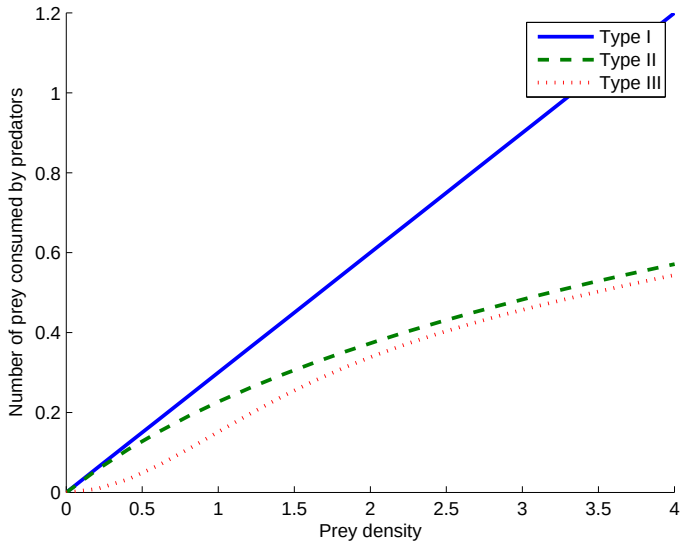


Figure 2.7: Graphical representation of Holling Type I, II and III responses.

equilibrium. For cyclic populations, the fitness equation no longer holds as it is only relevant for determining the evolution when a mutant is introduced to a resident population which is at equilibrium, hence we rely upon the results from the numerical simulations. Using this approach, it is clear that the general trends hold. We see that there is no significant difference by altering the functional response as the results are similar to those from the Type I model (Figure 2.5i). Figure 2.8 shows that in both cases, for f greater than zero, the hosts maximise their resistance at intermediate values of c where the susceptible and infected hosts coexist with the predator population. For f close to zero, the initial part of the curve changes as it did for the Type I model.

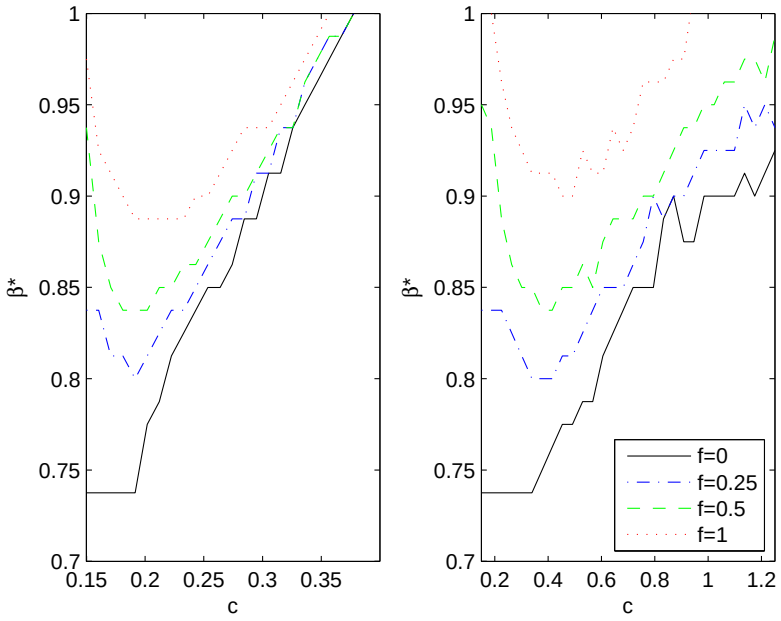


Figure 2.8: CSS transmission rate (β^*) for varying f for Holling Type II and III responses (result of running 30 simulations in which the mutant is introduced at an arbitrary point within the population cycle to solve for β^*, see Box 2.6 for how numerical simulations are run), where $a''(\beta) = -0.8$ and $h_S, h_I = 1.5$. Other parameters are given in Table 2.1. Here population cycles arise for $c > 0.2$ (see Box 2.1) and fitness is averaged over a finite run of mutant dynamics leading to the jagged lines (running over a longer time period may improve the smoothness of the curve).

Box 2.5: Holling Type II and III models

The fitness equation for the Type II model is found using the same method as the Type I model and is given by:

$$s(\hat{a},\hat{\beta};a,\beta) = (\hat{a} - qH - b - \hat{\beta}I - \frac{cP}{S+h_s})(\Gamma + \frac{c\phi P}{I+h_i}) + \hat{\beta}I(\hat{a}f - qfH + \gamma)$$

The fitness gradient is given by:

$$\frac{\partial s}{\partial \hat{\beta}} \mid_{\hat{\beta}=\beta} = (a'(\beta) - I)(\Gamma + \frac{c\phi P}{I+h_i}) + I(af - qfH + \gamma) + \beta I f a'(\beta)$$

The fitness equation for the Type III model is given by :

$$s(\hat{a},\hat{\beta};a,\beta) = (\hat{a} - qH - b - \hat{\beta}I - \frac{xcS^{x-1}P}{S^x+h_s})(\Gamma + \frac{yc\phi I^{y-1}P}{I^y+h_i}) + \hat{\beta}I(\hat{a}f - qfH + \gamma)$$

The fitness gradient is given by:

$$\frac{\partial s}{\partial \hat{\beta}} \mid_{\hat{\beta}=\beta} = (a'(\beta) - I)(\Gamma + \frac{yc\phi I^{y-1}P}{I^y+h_i}) + I(af - qfH + \gamma) + \beta I f a'(\beta)$$

For the Type II and III cases, it becomes intractable to obtain explicit equilibrium population densities (additionally population cycles arise) so we run numerical simulations over a long period of time until the population cycles stabilise, rather than using the fitness equations as in the Type I case. By running simulations which include the mutant strains and the population dynamics, we can determine how evolution will occur. Repeating this for each of the c values produces the results shown in Figure 2.8 (where $x, y = 2$). See Geritz et al. (1998) for more details.

Box 2.6: Numerical simulations

Numerical simulations were carried out to determine the evolutionary behaviour of the host-parasite system over time. The numerical simulations for these models were run in Matlab. This method is particularly useful for when population cycles arise as the system is no longer at equilibrium making the fitness equation method (described in Box 1.1) inapplicable. For cases where population cycles arise, an arbitrary point within the population cycle is selected for which the evolutionary dynamics are then carried out. The models in Chapters 2 and 3 were set up with different types of hosts (with varying growth rates) and parasite with a generalist predator population $(S_1, ...S_N, I_1, ..., I_N, P)$, where S =susceptible hosts, I =infected hosts, P =predators and N =number of different types of hosts.

The system of ordinary differential equations was solved until the equilibrium was reached. At the end of each run, any strain below a density of 0.0001 was made extinct (density set to 0) and a mutation (mutated strain chosen by weighted density with equal probability of mutating up or down) was introduced at a low density. This process was repeated over a finite period of time to determine the outcome of the evolutionary dynamics, i.e. whether the mutant has invaded or not (Metz et al., 1992).

The numerical simulation for the model in Chapter 5 was set up with an intermediate host and parasites (with varying transmission rates) with a generalist predator $(S, I_1, ..., I_N, P_S, P_{I1}, ..., P_{IN})$, where S =susceptible hosts, I =infected hosts, P_S =susceptible predators, P_I =infected predators and N =number of different types of parasites.

2.3.6 Branching region

It has been shown previously that for values of c where the susceptible prey, infected prey and predator coexist, the size of the branching region (i.e. the range of trade-off curvatures that give branching) generally increases as c increases (Hoyle et al., 2012). For low c, the predator is excluded and for large c, once the population of infected prey is removed, there is no selection for resistance (β^* no longer exists). For intermediate c, where the susceptible prey, infected prey and predator populations coexist, the size of the branching region increases with c as shown in Figure 2.9. Below each curve lies the region that satisfies the relevant CS or ES condition. Below both lines represents a CSS point or an attractor, below the CS and above the ES lines represents a branching point, above the CS line represents a repellor and below the ES and above the CS lines represents a Garden of Eden point (numerical simulations showing branching and attractor points can be seen in Figure 2.11). Hence, the branching region occurs where it is CS and not ES (see Hoyle et al., 2012 for more on the general shape of these curves).

I examined the branching region, that is the range of trade-off curvatures at the singular point which will lead to evolutionary branching, for varying parameter values (Figure 2.10; see Box 2.7 for further explanation of the method used). The results obtained align with the previous finding as the range of trade-off curvatures (plotted along the y-axis in Figure 2.10) that give branching increased with c for regions where all three populations (S, I and P) are present. As ϕ and α increase, the range of c values (x-axis) for which there is branching decreases because the infected population is removed quicker (as shown in Figures 2.6a and 2.6b), however, the range of trade-off curvatures that give branching increases where all three (S, I and P) coexist. The parameters, θ and γ, show similar results to ϕ and α (see Figure 2.12). As d increases, the range of c values (x-axis) for which there is branching increases because the infected hosts remain in the population

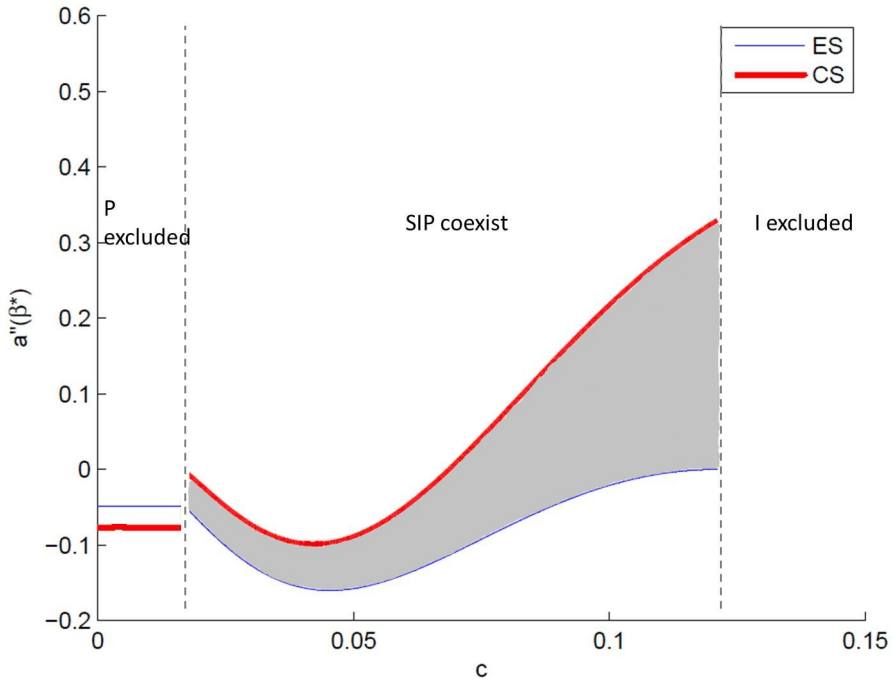


Figure 2.9: Branching region (shaded region) as the predation rate (c) varies, where $(\beta^*, a^*) = (1, 2)$. Other parameters are given in Table 2.1. The trade-off curvature ($a''(\beta^*)$) is plotted on the y-axis. The ES and CS conditions are plotted for the region where all three populations coexist (for low c, the predators are excluded and for high c, the infection is excluded).

for longer (as shown in Figure 2.6c), whilst the range of trade-off curvatures (y-axis) that give branching decreases when comparing the region where the susceptible prey, infected prey and predator populations coexist. The other parameters, q and b, show similar results to d (see Figure 2.12). Note that in all cases, branching tends to occur for close-to-linear trade-offs ($a''(\beta^*) = 0$).

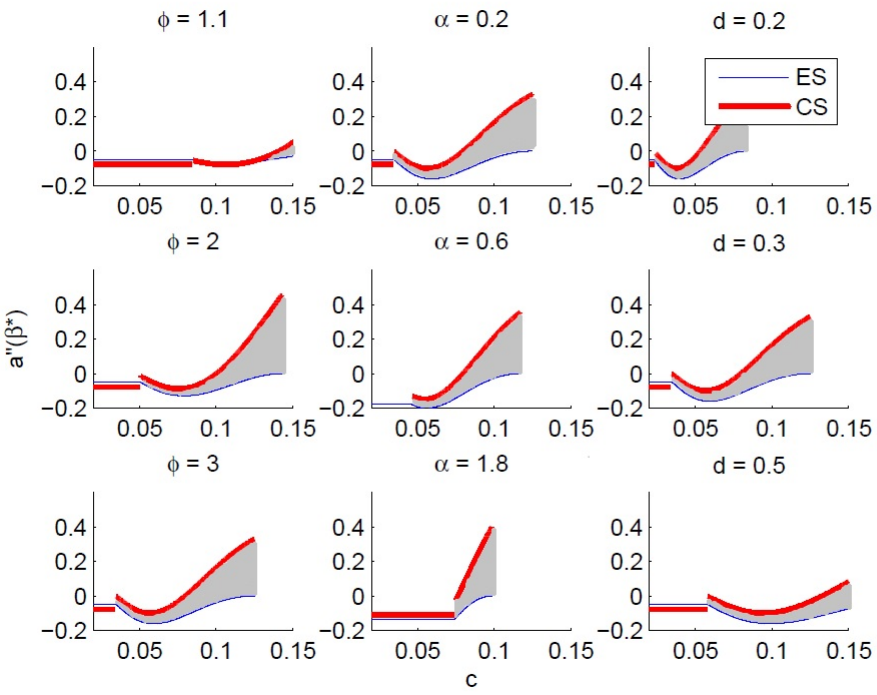


Figure 2.10: Branching region (shaded region) for varying parameters, where $(\beta^*, a^*) = (1, 2)$. Other parameters are given in Table 2.1.

Box 2.7: Stability conditions

The invasion boundary which determines when the mutant can and cannot invade the resident population, occurs when the fitness equation, $s(\hat{a}, \hat{\beta}; a, \beta) = 0$. Re-arranging this equation for $\hat{a}$ gives:

$$\hat{a} = qH + \frac{(b + cP)(\Gamma + c\phi P) + \hat{\beta} I(\alpha + b + c\phi P)}{\Gamma + c\phi P + f\hat{\beta} I} \tag{2.12}$$

Using this equation, we can compute the slope of the curve at the singular point:

$$\frac{\partial \hat{a}}{\partial \hat{\beta}} \mid_{\hat{\beta}=\beta^*} = \frac{I(\Gamma + c\phi P)(cP(\phi - f) + b(1 - f) + \alpha)}{(\Gamma + c\phi P + f\beta^* I)^2} \tag{2.13}$$

For analysis of the evolutionary outcome, we compute the following stability conditions. The ES (evolutionarily stability) condition is given by:

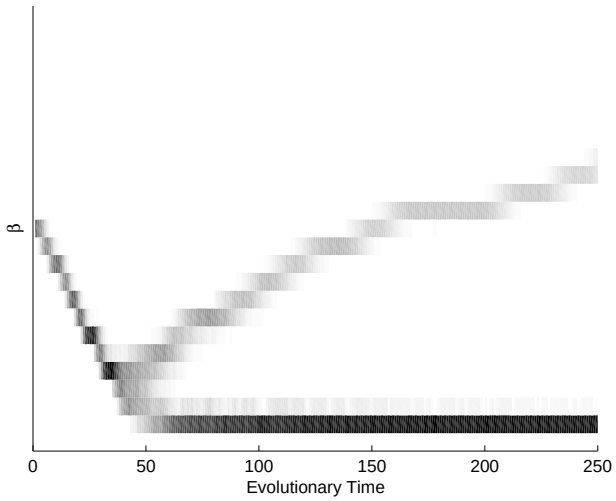
$$\frac{\partial^2 s}{\partial \hat{\beta}^2} \mid_{\hat{\beta}=\beta} = a''(\Gamma + c\phi P) + 2fIa' + f\beta I a'' < 0 \tag{2.14}$$

Here I use primes to denote derivatives, for example, $a' = \frac{\partial a}{\partial \beta}$. The MI (mutual invasibility) condition is given by:

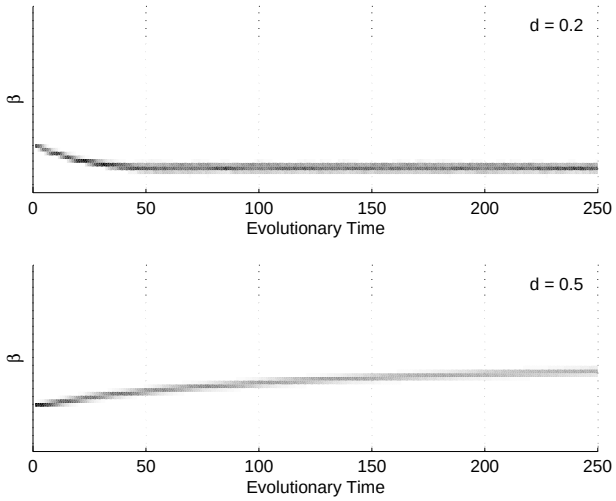
$$\frac{\partial^2 s}{\partial \hat{\beta} \partial \beta} \mid_{\hat{\beta}=\beta} = -\frac{\partial I}{\partial \beta}(\Gamma + c\phi P) + (a' - I)(c\phi \frac{\partial P}{\partial \beta}) + \frac{\partial I}{\partial \beta}(af - qfH + \gamma) - qfI(\frac{\partial S}{\partial \beta} + \frac{\partial I}{\partial \beta}) + f\beta \frac{\partial I}{\partial \beta} a' \tag{2.15}$$

The CS (convergence stability) condition is then given by $[\frac{\partial^2 s}{\partial \hat{\beta}^2} + \frac{\partial^2 s}{\partial \hat{\beta} \partial \beta}] \mid_{\hat{\beta}=\beta} < 0$ (ES + MI).

In my analysis, I fix the singular point at $(\beta^*, a^*) = (1, 2)$. I also fix all of the parameter values, except c. I then compute $a'(\beta^*)$ using equation 2.13 such that $(\beta^*, a^*) = (1, 2)$ is a singular point. I then find the values of $a''(\beta^*)$ for each value of c at which the ES and CS boundaries occur (i.e. where equation 2.14=0 and equations 2.14+ 2.15=0, respectively).

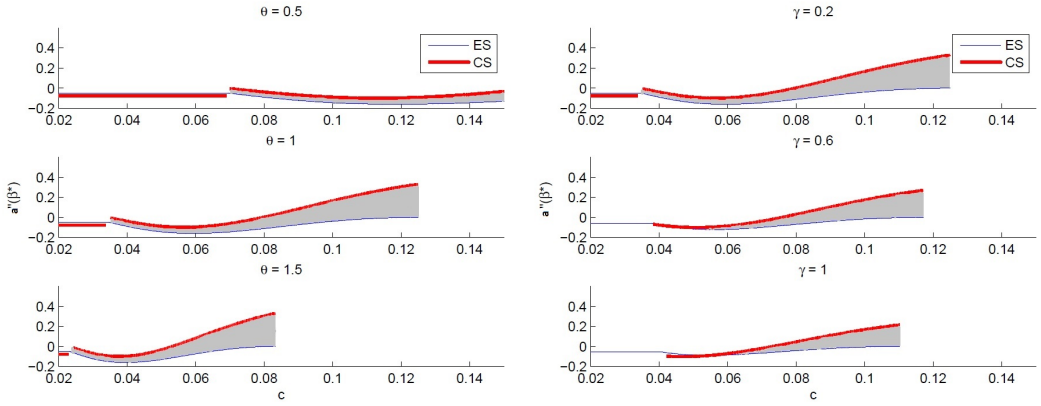


(a) Branching point, where $a''(1) = -0.05$.



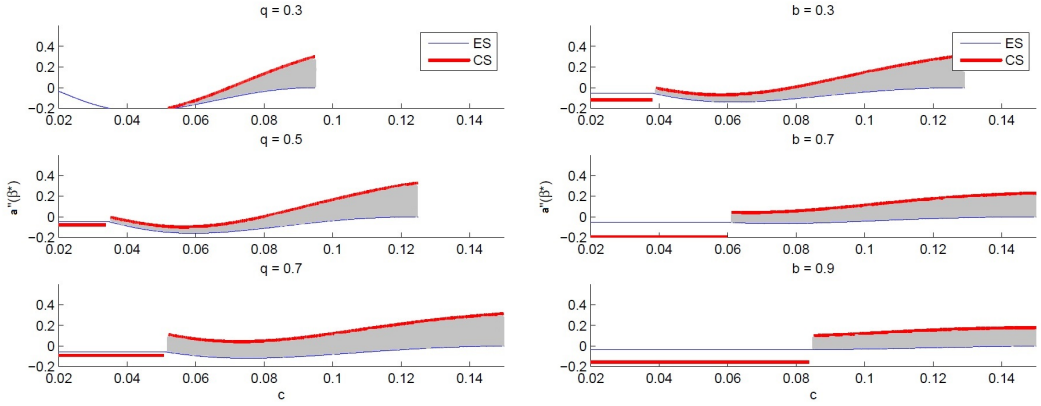
(b) High and low attractors for varying predator death rate (d), where $a''(1) = -0.15$.

Figure 2.11: Numerical simulations, where $c = 0.06$ and $a'(1) = 0.078$. Other parameters are given in Table 2.1.



(a) Varying θ.

(b) Varying γ.



(c) Varying q.

(d) Varying b.

Figure 2.12: Branching region (shaded region), where $(\beta^*, a^*) = (1, 2)$. Other parameters are given in Table 2.1.

2.4 Discussion

I have shown that the pressure from predation has important implications on the evolution of host defence to parasitism. I have found that selection for resistance to parasitism is greatest at intermediate predation rates, where the cost of infection due to predation is high but not enough to have led to a significant decrease in disease prevalence. I have also shown that, in re-

gions where the predator coexists with the susceptible and infected hosts, the predator increases the potential for diversity through evolutionary branching. Previous work on this model by Hoyle et al. (2012) focussed on the exclusion of populations and branching in the host. I have extended upon these findings by studying the impact of varying ecological parameters on the investment in host defence to determine how this is affected by predation. I have shown the population dynamics driving the evolution of host defence whilst providing more biological insight to explain the results. Additionally, I have shown how the branching region varies for these ecological parameters. My work highlights the importance of ecological feedbacks due to community interactions, and predation in particular, to evolutionary host-parasite interactions.

The results show that the predation rate has a significant impact on host defence levels. In general, hosts lower their defence if there is a low *cost* of being infected due to low predation, or a low *risk* of infection due to low disease prevalence. At low predation rates, any increase in predation leads to an increase in defences because there are still many infected hosts in the population so there is a high risk of becoming infected, as well as an increasing cost of infection due to predator-related mortality. However, at high predation rates, any further increases lead to lower defences because, despite the increasing cost of infection, most of the infected hosts have been removed through predation so there is a low risk of becoming infected. Therefore, at an intermediate predation rate, the hosts maximise their defence as there are many infected hosts (high risk) and a growing predator population (high cost).

In other host-parasite evolutionary studies without predation, it has been found that higher virulence generally selects for lower defence because of decreased exposure risk (Boots and Haraguchi, 1999). As we consider a predator in our system, we found this to be true for high predation rate values, where there is a low risk and high cost of being infected, but this result

changed for lower predation rate values. At low and intermediate predation rates, we found that initially as virulence increased, the hosts increased resistance due to the high cost and risk of being infected. So as virulence increased, the maximum investment in defence was higher. At high predation rates, although the cost of infection is high, the exposure risk drops due to the reduction in the infected host population, so here we see that higher virulence selects for lower defence. From these earlier studies, we would expect the virulence results to be most similar for low predation rates, rather than high predation rates, hence, this reveals a more subtle evolutionary effect of predation and the importance of feedbacks. In another non-predator study, Miller et al. (2007) found that resistance was not beneficial to the host when there was a large infected population because the host is likely to become infected regardless of their defence mechanisms. However when considering the addition of a predator population, we see that investment in defence may be beneficial if there is a large infected *and* predator population giving a high risk *and* cost of infection. Therefore, we see that the presence of a predator in the population can change the evolution of host resistance.

As the infected fecundity, the ability of infected hosts to reproduce, approaches zero in the Holling Type I, II and III response models, at low predation rates, the hosts no longer need to build up their levels of resistance due to the low numbers of infected hosts and predators in the population. As the predator population grows, with an increasing predation rate, the infected population becomes even smaller leading to a low risk of infection so the hosts lower their defences and focus on their birth rate. When the infected hosts are able to reproduce, however, the hosts maximise their levels of resistance at an intermediate predation rate. These results hold regardless of the Holling Type response used in the model and of whether the predator prefers to consume the susceptible prey, infected prey or both equally, as the hosts continually maximise their defences at an intermediate predation rate where the susceptible and infected hosts coexist with the predator population.

The addition of the predator increases the potential for evolutionary branching in the model, as also found by Hoyle et al. (2012). The results show that when the susceptible prey, infected prey and predators coexist, the range of trade-off curvatures that give branching increases as the predation rate increases. Here we have extended these results to show that as the virulence, the predation rate suffered by infected individuals, the conversion of predation into births of new predators or the recovery rate increase, the range of predation rate values for which there is branching decreases. This is because the infected population is removed quicker, although, the range of trade-off curvatures that give branching increases where S, I and P coexist. On the other hand, as the rate of density-dependent competition, the natural prey death rate or the predator death rate increase, the range of predation rate values for which there is branching increases because the infected population remains in the population for longer. Morozov and Best (2012) similarly found that the addition of a predator creates the potential for evolutionary branching in parasite virulence, rather than classic R_0 maximisation (Bremermann and Pickering, 1983, Bremermann and Thieme, 1989). In general, therefore, incorporating predators into host-parasite models appears to increase the possibility of coexistence and diversity of strains within communities. It would be interesting to explore whether community interactions per se lead to greater diversity in host-parasite systems or whether less antagonistic interactions, such as mutualisms, do not promote more diversity.

There are a range of interactions between hosts, parasite and predators in natural systems (Hatcher et al., 2006). My focus has been on predators that selectively predate on infected hosts (i.e. $\phi > 1$ in our model), although I found that the results held for cases where uninfected hosts experience greater predation. An example of this type of interaction includes a population of two salmon species (pink salmon and chum salmon) with a common predator, where sea lice (generalist parasite) can lead to reduced predation of chum salmon due to the predators preference to consume pink salmon (Peacock

et al., 2013). Therefore, as the parasite increases the predation risk for the preferred species, it lowers the predation risk for the other. However, when the host population is small, the parasite leads to increased predation of both host species as the predators can no longer be so selective (Peacock et al., 2013). The presence of an alternative prey species for the predator population may lead to the predator switching between prey, potentially with a preference for the species which is infected. Since the cost of infection is dependent on the density of infected prey, this non-linear variation may lead to a situation mimicked by the Type III response. Furthermore, if the predator can switch prey when the focal host reaches low densities, we would expect the predator to persist for longer. However, further analysis would be needed to fully determine the effect of an additional prey species on the evolutionary outcome.

Cases of apparent competition may also be seen in nature if there are two host species which share a generalist parasite and within-population phenotypic variability may also occur, as some individuals may be more or less susceptible to parasites (Hassell, 2000). Another source of complexity in natural systems is when the prey acts as the intermediate host for the parasite. In this case, the parasite may manipulate their host's behaviour to make them conspicuous to predators. For example, the "brain worm" parasite, *Dicrocoelium dendriticum*, manipulates ants foraging behaviour making them more prone to predation (Spindler et al., 1986, Moore, 2002). We consider this further in Chapter 5. Competition can also arise between predators, for example, in lions there is competition if they are hunting in the same location and there is a limited supply of prey (Schaller, 1972). While my key results appear to hold for some of these interactions, further analysis is needed for a fuller understanding of community interactions on disease dynamics.

More analysis into the complex population dynamics between hosts, parasites and predators is important as it will increase our understanding of how these populations evolve in nature. I have shown that the predator can

change the evolutionary behaviour by impacting the exclusion thresholds, stability and branching possibilities in a host-parasite system. Further studies in this area will help uncover methods which could be used to assist in the conservation and management of ecosystems.

Chapter 3

The evolution of host defence against multiple enemy populations

3.1 Introduction

In Chapter 2 we considered a host population evolving defence mechanisms against a single enemy population, parasites, however as many ecological populations are faced with a wide range of natural enemies, we can consider a host population able to evolve defence mechanisms against multiple enemy populations. Often adaptations to one enemy will also confer an advantage against others, for example a behavioural change may reduce encounters with multiple enemies (Moore, 2002). However, given limited resources, we may often expect improved defence against one enemy to constrain defence against another. Such a trade-off has been demonstrated experimentally in a number

Parts of this chapter are in the paper (see Toor and Best, 2016) published in the *American Naturalist*.

of systems, for example in bacteria against phage and protists (Friman and Buckling, 2012), in a plant against two herbivores (Stinchcombe and Rausher, 2001) and in flies against parasitic wasps and birds (Craig et al., 2007). Given these examples of antagonistic community interactions, it is important to understand the factors that impact the evolution of host defence strategies against their different enemies.

From a theoretical perspective, we now have a considerable understanding of how the underlying ecology can drive the evolution of host defences against a single natural enemy. A key example is the evolution of costly host defence against parasites (see review by Boots et al., 2009). If we consider the direct impacts of additional natural enemies, we may expect it to primarily lead to increased host mortality. Miller et al. (2007) explored how varying mortality rates impact a range of host defence mechanisms, finding that shorter-lived hosts generally invest less in defence to disease as they are less likely to become infected, although the results are complicated when models include acquired immunity (Miller et al., 2007, Best and Hoyle, 2013). However, considering increased mortality alone does not account for the dynamic feedbacks that result from the inclusion of a second enemy. We may also expect infected hosts to be preferentially targeted by additional enemies, with empirical evidence from red grouse (Hudson et al., 1992) and snowshoe hare (Murray et al., 1997) populations, such that infected hosts have higher mortality.

Theoretically, it has been shown that sterilising diseases which incur higher parasite-induced mortality (virulence) should select for lower host resistance due to the reduction in disease prevalence (Boots and Haraguchi, 1999), while van Baalen (1998) found that resistance through increased clearance is maximised at intermediate rates of virulence. It is also well known that disruptive selection leading to the coexistence of host strains (evolutionary branching) can occur when host defence is through resistance, but not when defence is through tolerance (Boots and Bowers, 1999, Roy and

Kirchner, 2000, Miller et al., 2005 but see Best et al., 2008) and Bruns et al. (2014) found that polymorphisms occurred in long-lived hosts for more costly and more extreme resistance levels, compared to short-lived hosts. Although these studies give us an indication of host defence against parasites, they do not consider the evolution of host defence when there is an additional enemy present.

Another well studied exploiter-victim interaction is predator-prey systems, with the focus often on the potential for Red Queen co-evolutionary cycles (Marrow et al., 1992, Dieckmann et al., 1995) or on the occurrence of diversity through evolutionary branching (Day et al., 2002, Abrams, 2003, Geritz et al., 2007, Hoyle and Bowers, 2007, Landi et al., 2013). Landi et al. (2013) found that prey branching leading to dimorphism is induced when prey are highly sensitive to competition as this increases the advantage of branching into prey with distinct traits. More generally, it has been found that the evolution of antipredator defence in the prey can promote the coexistence of two prey species with different traits and a predator population (Yamauchi and Yamamura, 2005). However, the effects of additional species interactions have also not been included in these studies.

Recently, there has been a growing interest in the dynamic effects of predation on host and parasite evolution (Morozov and Adamson, 2011, Hoyle et al., 2012, Morozov and Best, 2012, Kisdi et al., 2013). Specifically to host evolution, in Chapter 2, I showed that given a trade-off between host investment in defence against infection and birth rate, host investment in defence to disease is maximised at intermediate predation rates, i.e. intermediate additional mortality, where there is both a high risk and cost of infection, with this effect being heightened when there is strong selective predation on infected hosts (in contrast to Miller et al., 2007 which looked at host defence against parasites alone and found that defence is highest when mortality is low). Meanwhile Hoyle et al. (2012) showed how the presence of a predator can lead the host to evolve such that there is deterministic eradication of

the disease, which cannot occur in a standard host-parasite model. Both of these studies also showed that the presence of a predator population increases the parameter range leading to evolutionary branching in host defence (Hoyle et al., 2012 and Chapter 2). For parasite evolution, Morozov and Best (2012) showed that the additional feedbacks from the predator can lead to branching and coexistence of multiple parasite strains, whilst Kisdi et al. (2013) have shown that cyclic fluctuations of parasite virulence and predator densities can arise. Predation of hosts therefore appears to make the diversity of hosts and parasites more likely. Although these studies have provided useful insight into host-parasite evolution in the presence of a predator population, they still do not directly address the question of what happens when host species must allocate resources between defences against infection and predation.

In this chapter, I focus on a host population facing two enemies, parasites and predators. I assume that by increasing defence to one enemy, the host constrains their defences against the other. Using an evolutionary invasion (adaptive dynamics; Geritz et al., 1998) approach, I look at the evolutionary behaviour of the host population with the aim of determining when the hosts are more likely to defend themselves against the infection or predation.

3.2 Model

We modify the classic host-parasite model used by including an additional predator population (as used in Hoyle et al., 2012 and Chapter 2 but with a Holling Type I response and a different trade-off between the predation and transmission rates, $c(\beta)$, to focus on host defence against the two enemies).

Table 3.1: Parameter definitions and values used

Parameter	Definition	Default Value
β	Transmission rate	Varies
a	Prey birth rate	2
α	Parasite-induced death rate/virulence	1.3
ϕ	Increase/decrease in the predation rate suffered by infected individuals	3
θ	Conversion of predation into births of new predators	1
γ	Recovery rate	0.2
q	Rate of density-dependent competition, acting on births	0.5
b	Natural prey death rate	0.2
d	Predator death rate	0.3
f	Infected fecundity (proportion of infected individuals able to reproduce)	1
Γ	1/Infectious period, $\alpha + b + \gamma$	0.6
c	Predation rate	Varies

The model is given by the following ordinary differential equations:

$$\frac{dS}{dt} = a(S + fI) - qH(S + fI) - bS - \beta SI + \gamma I - cSP \tag{3.1}$$

$$\frac{dI}{dt} = \beta SI - (\alpha + b + \gamma)I - c\phi IP \tag{3.2}$$

$$\frac{dP}{dt} = \theta cP(S + \phi I) - dP \tag{3.3}$$

where S, I and P are the densities of susceptible hosts, infected hosts and predators, respectively with $H = S + I$. The parameters are defined in Table 3.1 (parameter values selected as this is a region where S, I and P coexist). Refer to Figure 2.1 in Chapter 2 for a schematic diagram of the model. In this model, once a host is infected it moves from the susceptible class to the infected class. If the infected host recovers, it moves back into the susceptible class as it can be re-infected by the parasite (susceptible-infected-susceptible [SIS] model). Using a Holling Type I response, I assume that there is no limitation on predation (in Chapter 2 I found that the evolutionary behaviour qualitatively holds for Type II and III responses provided that the population dynamics remain as equilibria). I also assume that the predator is immune to infection in this system.

Additionally, I include a trade-off, $c(\beta)$, between the predation rate (c) and transmission rate (β) in the model different to the previous trade-off used in Chapter 2 as here the trade-off is between predation rate, rather than prey birth rate. The trade-off is given by (similar form to that previously used by Hoyle et al., 2012 and in Chapter 2):

$$c(\beta) = c(\beta^*) - \frac{c'(\beta^*)^2}{c''(\beta^*)}\left(1 - e^{\frac{c''(\beta^*)(\beta-1)}{c'(\beta^*)}}\right) \tag{3.4}$$

Here I use primes to denote derivatives, for example, $c'(\beta^*) = \frac{\partial c}{\partial \beta}\big|_{\beta=\beta^*}$ and (β^*, c^*) is the evolutionary singular point. For my results, I set the singular point, gradient and curvature of the trade-off curve as $(\beta^*, c^*) = (1.15, 0.08)$, $c'(\beta^*) = -0.2$ and $c''(\beta^*) = 1$, respectively (values selected as this is a region where the S, I and P populations coexist). Given that $c''(\beta^*) > 0$, the trade-off curve is a decreasing function with resistance becoming increasingly costly (concave curve) as shown in Figure 3.1. The mutant strains vary in their strategies along this trade-off, such that higher host defence against one enemy corresponds to lower defence against the other. If $c''(\beta^*) < 0$, the curve switches to one with decelerating costs (convex curve).

In this case ($c'(\beta^*) < 0$), when the hosts increase defence against one enemy (the parasite or the predator), they constrain their defence against the other. As the hosts increase their resistance against the infection, they lower their resistance against predation (β decreases and c increases), whereas, as the hosts increase their resistance against predation, they lower their resistance against the infection (c decreases and β increases). For example, evolving towards low c will lead to β increasing as the hosts are defending themselves against predation, rather than infection. There is good experimental evidence that such a trade-off exists (Stinchcombe and Rausher, 2001, Nuismer and Thompson, 2006, Craig et al., 2007, Edeline et al., 2008, Gomez et al., 2009, Siepielski and Benkman, 2009, Friman and Buckling, 2012) but it is not clear theoretically when we would expect hosts to invest more in a

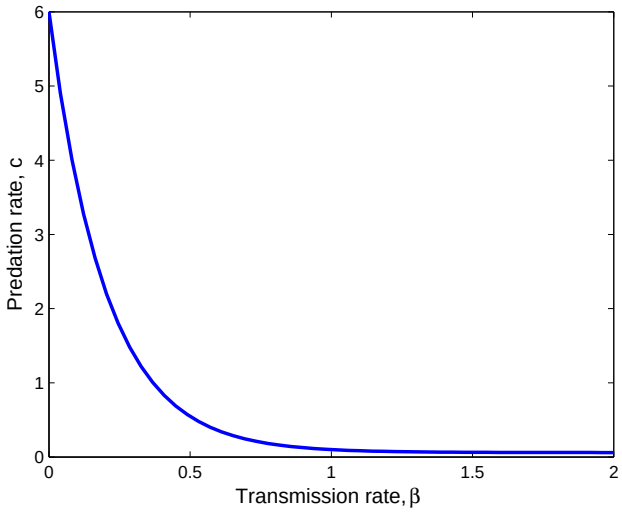


Figure 3.1: Trade-off curve between the predation rate (c) and transmission rate (β) where $(\beta^*, c^*) = (1.15, 0.08), c'(\beta^*) = -0.2$ and $c''(\beta^*) = 1$.

particular defence mechanism. I also consider the addition of another trade-off in the host in which their birth rate is lowered when defence against both parasites and predators is high (see Box 3.1).

I use the evolutionary invasion framework of adaptive dynamics (Geritz et al., 1998) to determine the evolution of host defence. This means that I introduce small, rare, nearby mutant strains to a resident strain at equilibrium. For this model, the fitness of a mutant host invading a resident equilibrium is given by the following sign-equivalent proxy fitness equation:

$$s(\hat{c}, \hat{\beta}; c, \beta) = (a - qH - b - \hat{\beta}I - \hat{c}P)(\Gamma + \hat{c}\phi P) + \hat{\beta}I(af - qfH + \gamma) \quad (3.5)$$

Here I use $\hat{c}$ and $\hat{\beta}$ to denote the mutant strain traits and assume that only the resident's density is affecting the mutant, given the rarity of the mutant (see Box 3.2 for the full derivation of the fitness equation). The fitness is equivalent to the mutant's growth rate whilst rare. When $s > 0$, the mutant population has positive fitness and can invade the resident population, and

when $s < 0$, the mutant population has negative fitness and cannot invade the resident population.

I determine the evolutionary outcome of the system by looking for evolutionary singular points which occur when the gradient of the fitness equation is zero:

$$\frac{\partial s}{\partial \hat{\beta}} \mid_{\hat{\beta}=\beta} = 0.$$

Continuously stable strategy (CSS) points are evolutionarily stable (ES, meaning the population will stay at this point once reached as no nearby mutants can invade) and convergence stable (CS, meaning the population will converge to this point when close to it). The ES and CS properties are met when certain second-order conditions are satisfied, see Section 3.3.6. I use the composition of the enemy communities (by obtaining the ratio of infected hosts to predators) to gain a better understanding of the mechanisms behind the evolutionary behaviour of the host population. I vary the parameters in the model to determine when the hosts are more likely to defend themselves against the infection or predation whilst focussing on the region where the susceptible and infected hosts coexist with the predator population (there are regions where the parasite, predator or both are excluded from the system). I then move on to look at the diversity that can be produced by looking at the possibility of the population branching into coexisting host populations with different defence strategies.

Box 3.1: Further trade-offs

Alongside the trade-off in evolving defences against the two enemies (shown in Figure 3.1), there is further experimental evidence that the host also faces a cost when evolving defences against both enemies (Friman and Buckling, 2012). When the host has high defences against both the parasite and predator populations, there is a cost as it minimises their birth rate. Hence, the hosts are able to maximise their birth rate by lowering their defences against the infection or predation. We include this additional trade-off in our model and look at how it impacts the evolution of host defence. This trade-off is represented by the following equation:

$$a = (2c - c_{min}) + (2\beta - \beta_{min}) \tag{3.6}$$

Including this trade-off in our model (along with the previous trade-off), I find that our main findings still hold, with the host shifting towards more defence against the parasite or the predator. It is more costly for the host to maintain defences against both the parasite and infection, so having greater defence against one of the enemies is more beneficial to the host. Including both trade-offs, $c(\beta)$ and $a(\beta)$, the gradient of the fitness equation is:

$$\frac{\partial s}{\partial \hat{\beta}} \mid_{\hat{\beta}=\beta} = (a'(\beta) - I - c'(\beta)P)(\Gamma + c\phi P) + (a - qH - b - \beta I - cP)(c'(\beta)\phi P) + I(af - qfH + \gamma) + \beta I a'(\beta) f \tag{3.7}$$

Comparing Figure 3.2 to Figure 3.3, for ϕ, we see that the host shifts towards increased defence against infection and lowered defence against predation because the ratio of infected hosts to predators has increased. Comparing Figure 3.2 to Figure 3.5 for α, we see that the host shifts towards increased defence against the disease and less defence against predation because of the increased risk of mortality once infected. Despite these shifts in greater defence against the infection or predation, our main findings remain the same with the inclusion of this trade-off.

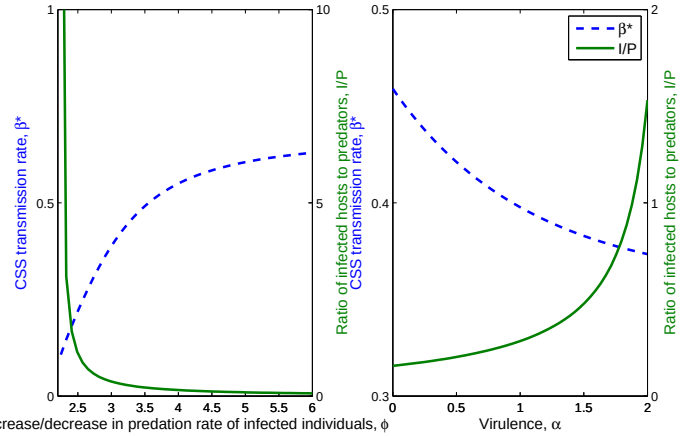


Figure 3.2: β^* and I/P for varying ϕ and α with additional trade-off in a, where $(\beta_{min}, c_{min}) = (0.4, 0.06)$. Other parameters given in Table 3.1.

Box 3.2: Fitness equation

To obtain the fitness equation for a mutant population trying to invade a resident equilibrium, I begin by considering a resident population which is at equilibrium and look at the possibility of invasion by a rare mutant. I use the stability of this resident-mutant equilibrium to calculate the fitness for the mutant population.

The following are the corresponding equations for the mutant population:

$$\frac{d\hat{S}}{dt} = a(\hat{S} + f\hat{I}) - q(\hat{S} + f\hat{I})H - b\hat{S} - \hat{\beta}\hat{S}I + \gamma\hat{I} - \hat{c}\hat{S}P \tag{3.8}$$

$$\frac{d\hat{I}}{dt} = \hat{\beta}\hat{S}I - (\alpha + b + \gamma)\hat{I} - \hat{c}\phi\hat{I}P \tag{3.9}$$

where $H = S + I$. The stability of the resident-only equilibrium depends on the eigenvalues from the mutant part of the resident-mutant system's Jacobian matrix, given by:

$$\begin{pmatrix} a - qH - b - \hat{\beta}I - \hat{c}P & af - qfH + \gamma \\ \hat{\beta}I & -\Gamma - \hat{c}\phi P \end{pmatrix}$$

where $\Gamma = \alpha + b + \gamma$.

The fitness equation is given by the dominant eigenvalue of this matrix. Alternatively, we take the negative determinant of the above matrix evaluated at the resident-only equilibrium, which gives:

$$s(\hat{c}, \hat{\beta}; c, \beta) = (a - qH - b - \hat{\beta}I - \hat{c}P)(\Gamma + \hat{c}\phi P) + \hat{\beta}I(af - qfH + \gamma) \tag{3.10}$$

This equation is sign equivalent to the fitness equation computed using the dominant eigenvalue of this Jacobian matrix (see Box 1.1 in Chapter 1 for proof). Hence I use this equation as a fitness proxy to determine the fitness in our analysis.

3.3 Results

I focus on the impact of the infected and predator populations on the evolution of host resistance whilst varying the parameters in the model to determine when the hosts are more likely to defend themselves against infection or predation. I focus on the region where the susceptible and infected hosts coexist with the predator population (there are regions where the parasite, predator or both are excluded from the system). In general, I find that the hosts increase their defences to either the infection or predation depending on the ratio of infected hosts to predators in the population. When there is a large infected population, the hosts increase their defences against the parasite as they are more likely to get infected, whereas, when there is a large predator population, the hosts increase their defences against the predators due to the higher risk of predation. Hence, the hosts tend to evolve their defences depending on whether they are more likely to get infected by a parasite or preyed upon by a predator, although this becomes more complicated when we include recovery and reproduction from infected hosts.

3.3.1 Proportion of infected hosts to predators

I begin by simplifying the model to assume that there is no recovery ($\gamma = 0$) and that the infected hosts are sterile ($f = 0$), such that the gradient of the fitness equation (equation 3.5) can be simplified to:

$$\left.\frac{\partial s}{\partial \hat{\beta}}\right|_{\hat{\beta}=\beta} = -\mu_I P\left(\frac{I}{P} + c'(\beta)\right) \tag{3.11}$$

where $\mu_I = \Gamma + c\phi P$ (rate at which infected hosts leave the infected compartment) and $c'(\beta) = \frac{\partial c}{\partial \hat{\beta}}\,|_{\hat{\beta}=\beta}$ (here $c'(\beta) < 0$). From equation 3.11, we see that at a singular point, $I/P = -c'(\beta)$, so any change in the ratio of the enemy populations will move the host population towards selection for lower or higher values of β. For example, if an environmental change leads to a larger

infected population and a smaller predator population, i.e. $I_{new} > P_{new}$, then the gradient will become negative leading to higher host resistance to the disease (lowering β). Clearly in this case the proportion of infected hosts to predators plays the key role in determining the direction of evolution of host defence against the infection or predation.

Considering the full model (where $\gamma > 0$ and $f > 0$), the gradient of the fitness equation (equation 3.5) is now given by:

$$\left.\frac{\partial s}{\partial \hat{\beta}}\right|_{\hat{\beta}=\beta} = P(\phi\rho_S - \mu_I)\left(\frac{I}{P}\frac{\rho_I - \mu_I}{\phi\rho_S - \mu_I} + c'(\beta)\right) \tag{3.12}$$

where $\rho_S = a - qH - b - \beta I - cP$ (contribution from susceptible hosts to the growth rate of the host population) and $\rho_I = af - qfH + \gamma$ (contribution from infected hosts to the growth rate of the host population). Now, since $\rho_s \leq 0$ and $\rho_I \geq 0$ at the singular point, the driver of selection is clearly no longer as simple as the ratio of infected hosts to predators, as now the growth rates also play a role. For example, if an environmental change leads to $P_{new} > I_{new}$, we also need to know what happens to the growth terms, ρ_S, ρ_I and μ_I. In fact, given the signs of the ρ_s and ρ_I terms at the singular point, even if $P_{new} > I_{new}$, the gradient may become negative leading to higher defence against infection.

In general, therefore, I find that the hosts increase their defences to either the infection or predation depending on the composition of the enemy populations (i.e. the ratio of infected hosts to predators present in the population). The hosts tend to evolve defence mechanisms against parasites or predators depending on which enemy has a higher population density as this relates to whether they are more likely to get infected by a parasite or preyed upon by a predator. Although, this may not always be the single driving factor of the hosts evolutionary behaviour as additionally the growth rates may play a role in determining the evolutionary behaviour of host defence.

Unless stated otherwise, I assume $\gamma > 0$ and $f > 0$ throughout the

following sections.

3.3.2 Predation rate suffered by infected individuals

I first consider how the degree of selective predation on infected hosts (ϕ) impacts host investment in defence mechanisms. As ϕ increases, there is an increasing risk of mortality through predation whilst infected. For $\phi > 1$, the predators are selectively preying upon infected hosts so as ϕ increases, (provided that there is a sufficient amount of infected hosts available for the predators to consume) the rate of growth of the predator population increases and removes the infected hosts from the population thereby lowering the risk of infection for the susceptible hosts. For high virulence (α), as ϕ increases, the ratio of infected hosts to predators decreases because the infected hosts are being removed through predation and parasite-induced mortality (see solid line in Figure 3.3), giving a relatively lower chance of recovery or reproduction whilst infected, therefore the dynamics become similar to an SI system. Hence, the predators pose a greater threat upon the host population which leads to the hosts increasing their resistance to the predators whilst consequently becoming more susceptible to infection (β^* increases as shown by the dashed line in Figure 3.3 for high α and Figure 3.4). The parameters for the prey birth rate (a) and the conversion of predation into births of new predators (θ) behave similarly to ϕ as an increase in these parameters also leads to the host population increasing their defences against predation (similar to the dynamics shown in Figure 3.3 for $\alpha = 0.8$).

This result changes for low virulence (α) values. In this case, as ϕ increases, the hosts initially increase their defences against predation then switch to increase their defences against infection (see the dashed line in Figure 3.3 for low α and Figure 3.4). Since α is small (here, it is more likely that infected hosts will be able to recover or reproduce), the ratio of infected hosts to predators is no longer the single factor determining the evolution of host defence (here $\rho_I - \mu_I$ changes from positive to negative, see

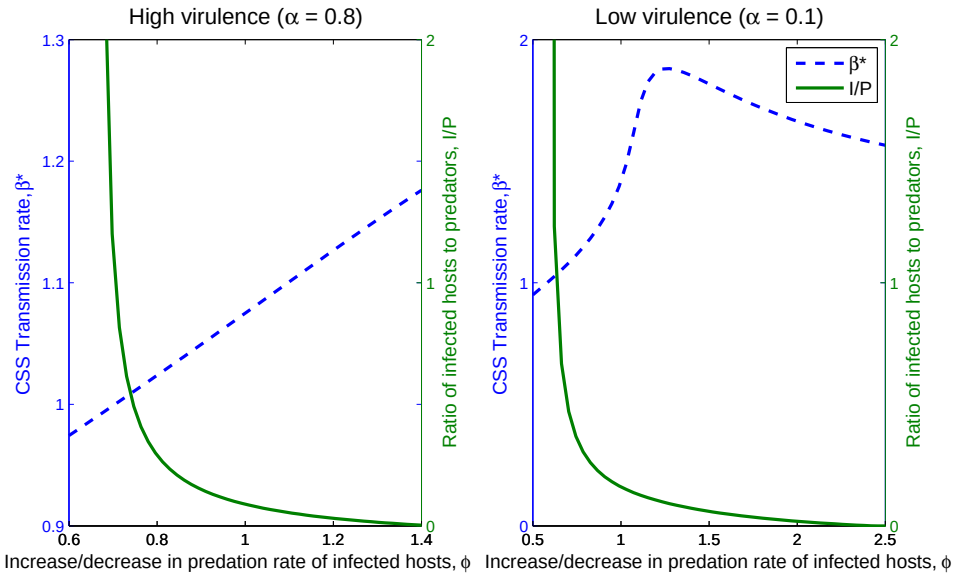


Figure 3.3: CSS transmission rate (β^*) and ratio of infected hosts to predators (I/P) for varying ϕ, where $d = 0.22, \beta = 1, c = 0.1$ and other parameters are given in Table 3.1 (the results hold for higher ϕ values using all of the parameter values given in Table 3.1).

equation 3.12). As ϕ increases, it becomes increasingly risky to become infected as the host is more likely to be preyed upon once infected so the hosts switch to defending themselves against infection, even though the predator is the main enemy. Hence, for less virulent diseases, as ϕ increases, the hosts increase their defences against predation then switch to increase defences against infection.

3.3.3 Virulence

The hosts increase their defences against the infection as virulence (α) increases (this holds for all values of $0 \leq f \leq 1$). Here the predators have less infected hosts to prey upon because the parasite is removing infected hosts quickly from the population so the predator population decreases and the

ratio of infected hosts to predators increases (see solid line in Figure 3.5). The infected population is also decreasing as α increases but it is still larger than the predator population. Hence, due to the increased parasite-induced mortality, the parasite poses a greater threat upon the host population so the hosts correspondingly increase their resistance to the parasite (see Figures 3.4 and dashed line in 3.5).

I find that this also holds when the predators are selectively preying upon the susceptible hosts, showing that increasing α has a highly detrimental effect on the predator population as the predators do not have enough susceptible hosts to maintain their population density. Hence, for varying values of ϕ, I find that the hosts continually increase resistance to the disease as α increases, regardless of whether the predators selectively prey upon the susceptible hosts, infected hosts or both equally. The parameters for the rate of density-dependent competition acting on births (q), natural prey death rate (b) and predator death rate (d) behave similarly to α (Figure 3.5). Increases in q and b lead to a smaller host population so the predator population declines as they have less prey to consume causing the hosts to increase their defences against the infection as it poses a larger threat. An increase in d leads to a decline in the predator population so the hosts once again focus on defences against the infection, rather than predation.

3.3.4 Recovery rate

As the recovery rate (γ) increases, the infected hosts are recovering and returning to the susceptible class at a quicker rate. For γ, the results change depending on whether the predators selectively prey upon the susceptible hosts, infected hosts or both equally. For values of ϕ close to 1, the predators are being less selective when deciding which prey to consume so both the susceptible and infected hosts are being preyed upon. As γ increases, the number of infected hosts decreases as they are recovering from the disease and re-entering the susceptible class, lowering the risk of infection. This causes

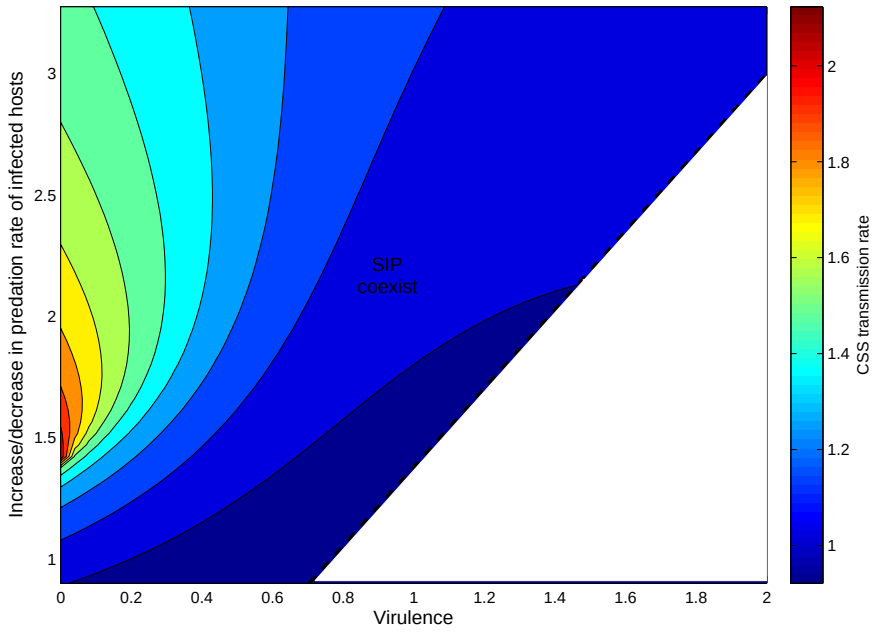


Figure 3.4: CSS transmission rate (β^*) for varying α and ϕ, using the parameter values given in Table 3.1. The susceptible and infected hosts coexist with the predator population in the region to the left of the dashed line (the predator or both the predator and parasite population have been excluded in the white region).

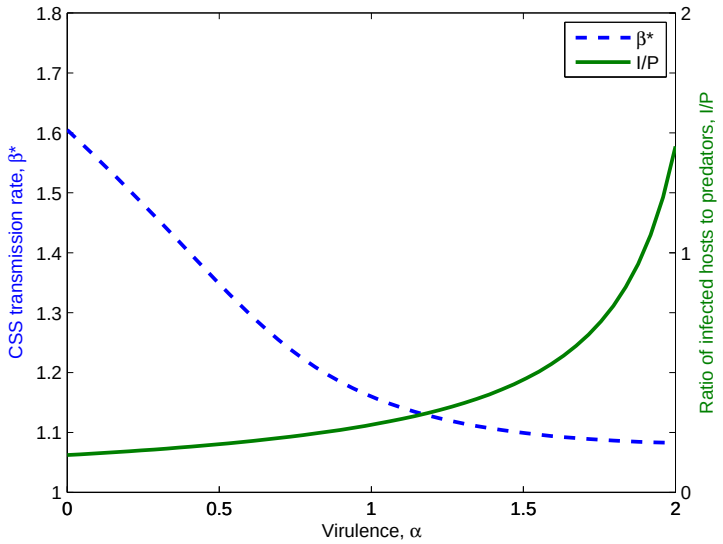


Figure 3.5: CSS transmission rate (β^*) and ratio of infected hosts to predators (I/P) for varying α values, using the parameter values given in Table 3.1.

a reduction in the ratio of infected hosts to predators so the hosts increase their defences to the predators as they face a higher risk of predation, rather than infection (Figure 3.6).

For higher values of ϕ, as γ increases, the hosts first increase resistance to the predators then to the infection (β^* increases then decreases as shown in Figure 3.6). Initially as γ increases, there are less infected prey leading the hosts to increase their defences against predation. However, the ratio of infected hosts to predators begins to increase because the predators are more reliant upon the infected population and have less infected hosts to consume as more of them are recovering so the predator population declines. As the pool of susceptible hosts increases, this eventually leads to an increase in the number of infected prey because there are more susceptible hosts for the parasite to infect. The hosts then switch their defence strategy and focus on increasing their defences against the infection for large values of γ. For low ϕ, this increase in γ does not lead to an increase in the susceptible pool because the predators are being less selective and are also preying upon the susceptible hosts (rather than selectively preying upon infected hosts, which is the case when ϕ is large). Hence, as γ increases, the hosts increase their defences against the predators when ϕ is low and against the infection when ϕ is high.

3.3.5 Infected fecundity

As the infected fecundity (f) approaches zero, there is a smaller proportion of infected individuals which are able to reproduce and when $f = 0$, the infected population is completely sterile. The infected hosts only reach a small density as f approaches zero so it takes a longer time for the predator population to grow as there are less prey for them to consume. When f is large, the predator population has more infected hosts to consume so the predator population grows and the infected population decreases, leading to the predators posing a greater threat upon the susceptible host population.

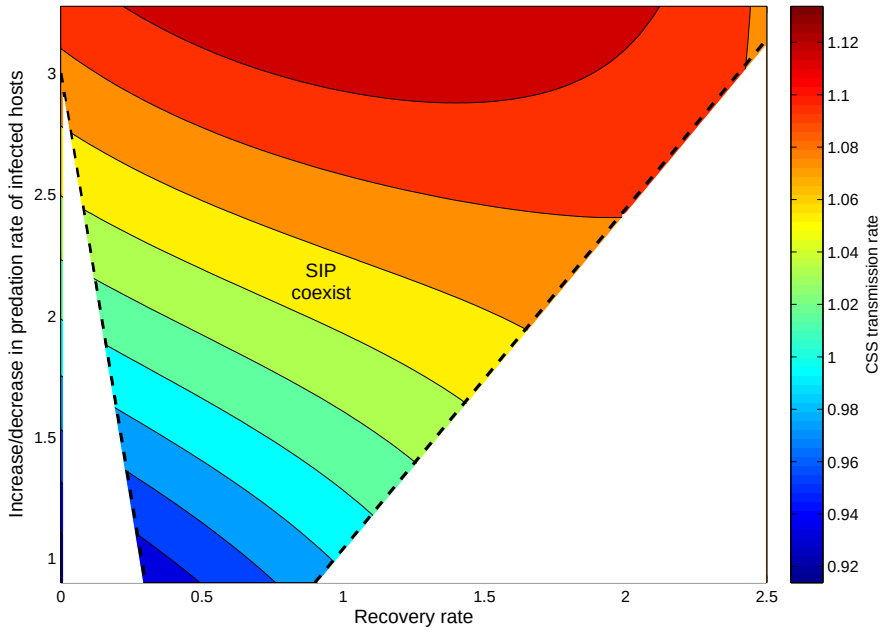


Figure 3.6: CSS transmission rate (β^*) for varying γ and ϕ, using the parameter values given in Table 3.1. The susceptible and infected hosts coexist with the predator population in the region between the two dashed lines (the predator population has been excluded for low γ and the parasite population has been excluded for high γ in the white regions).

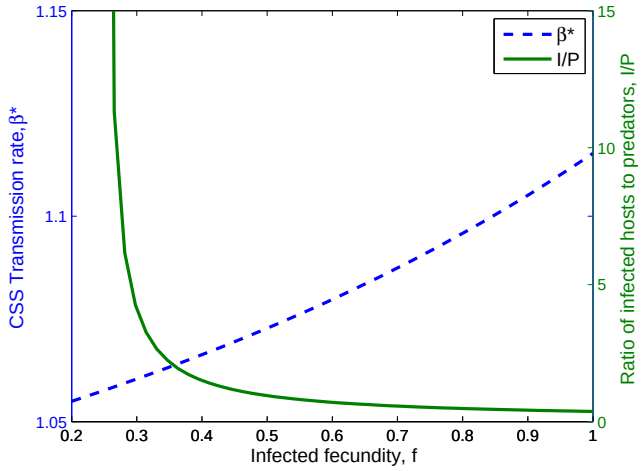


Figure 3.7: CSS transmission rate (β^*) and ratio of infected hosts to predators (I/P) for varying f, using the parameter values given in Table 3.1.

This decrease in the ratio of infected hosts to predators as f increases is shown in Figure 3.7 (solid line). Hence, as f increases, the hosts increase their defences to the predators, becoming more susceptible to the infection, so β^* increases as shown in Figure 3.7 (dashed line).

3.3.6 Branching region

Next, I looked at the possibility of two populations coexisting through the occurrence of disruptive selection (evolutionary branching) in the system. Here, I use the predator death rate (d), to illustrate the branching results as this parameter has a clear direct effect on the predator population density whilst leaving the other parameters free to vary. Figure 3.8A shows the regions where various evolutionary outcomes can occur at a fixed singular point. For low d values, the infection is excluded and for high d values, the predators are excluded. Above each curve lies the region that satisfies the

relevant ES or CS condition, equation 3.13 or equation 3.14, respectively.

$$\frac{\partial^2 s}{\partial \hat{\beta}^2} \mid_{\hat{\beta}=\beta} < 0 \tag{3.13}$$

$$\left[\frac{\partial^2 s}{\partial \hat{\beta}^2} + \frac{\partial^2 s}{\partial \hat{\beta} \partial \beta}\right] \mid_{\hat{\beta}=\beta} < 0 \tag{3.14}$$

Above both lines represents an attractor (CSS), above the CS and below the ES lines represents a branching point, below the CS line represents a repellor and above the ES and below the CS lines represents a Garden of Eden point (a locally repelling fitness maximum, i.e. these are points which the population will not evolve towards but populations at these points will stay there). The branching region occurs where it is CS and not ES. Along the y-axis is the curvature of the trade-off at the singular point which determines the behaviour at the evolutionary singularity (the slope of the trade-off curve determines whether the point is an evolutionary singularity). Figure 3.8B shows a numerical simulation where the host population converges to a point then undergoes disruptive selection and branches into two coexisting populations with varying defence strategies. Here we get two specialist host populations: one host population evolves low defences against the disease (population evolving towards high β) with high defences against predation and the other evolves high defences against the disease (population evolving towards low β) with low defences against predation.

Figure 3.9 shows the branching region for varying values of ϕ. The ES and CS conditions are only plotted for the parameter space where all three species coexist. For low d values, the infection is excluded and for high d, the predators are excluded. I find that as ϕ increases, the size of the branching region (i.e. the range of trade-off curvatures that give branching) decreases but note that the range of d values for which there is branching increases. Branching is more likely for lower values of ϕ because the hosts are more likely to defend themselves against the disease or predators as both are

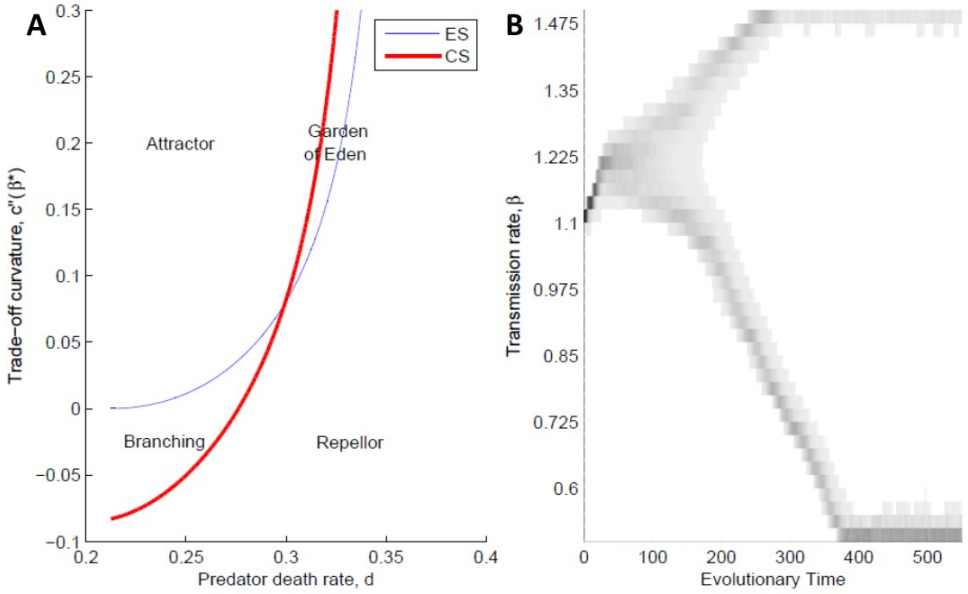


Figure 3.8: A) Various regions for differing evolutionary outcomes, where parameters are given in Table 3.1. Above each curve lies the region that satisfies the relevant evolutionarily stable (ES) or convergence stable (CS) condition. B) The numerical simulation shows a host population branching into two coexisting populations, where $\beta_{min} = 0.5, \beta_{max} = 1.5, c''(\beta^*) = -0.05, d = 0.25$ and other parameters are given in Table 3.1.

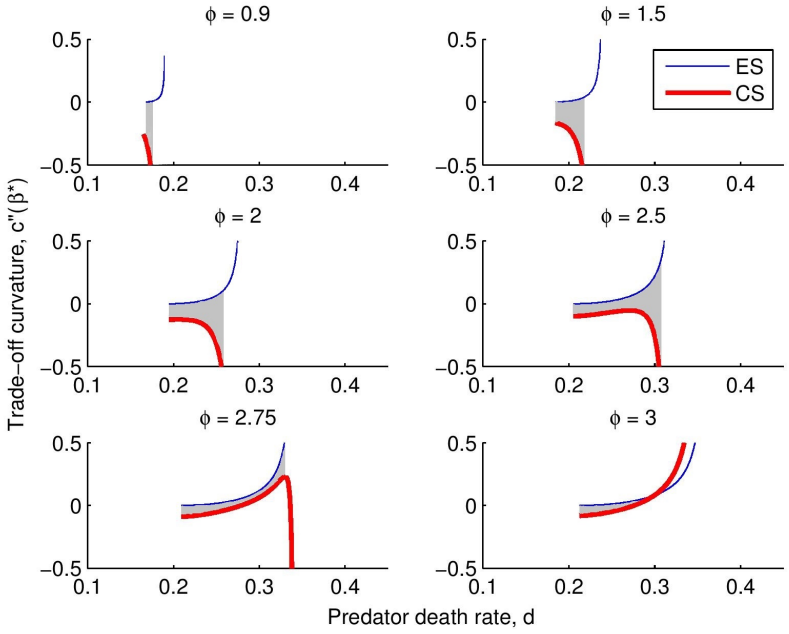
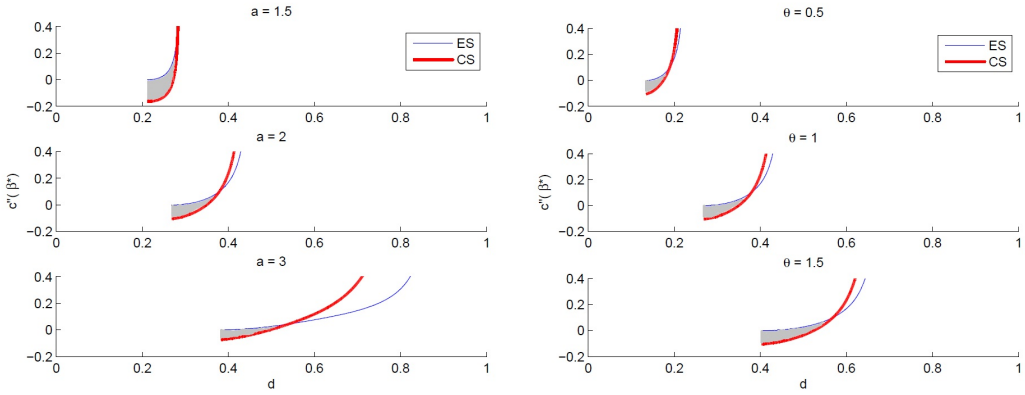
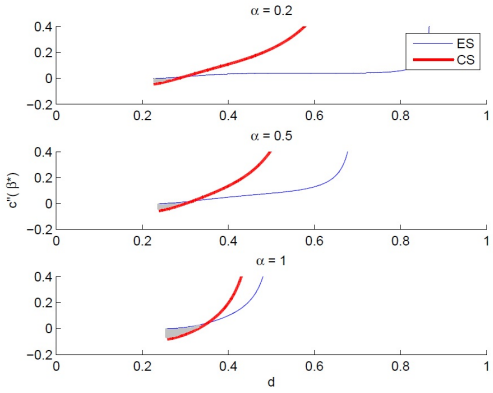


Figure 3.9: Branching region (shaded region) for varying parameters, where $(\beta^*, c^*) = (1.15, 0.08)$. Other parameters are given in Table 3.1. The trade-off curvature ($c''(\beta^*)$) is plotted on the y-axis. The evolutionarily stable (ES) and convergence stable (CS) conditions are plotted for the region where all three populations coexist (for low d values, the infection is excluded and for high d values, the predators are excluded).

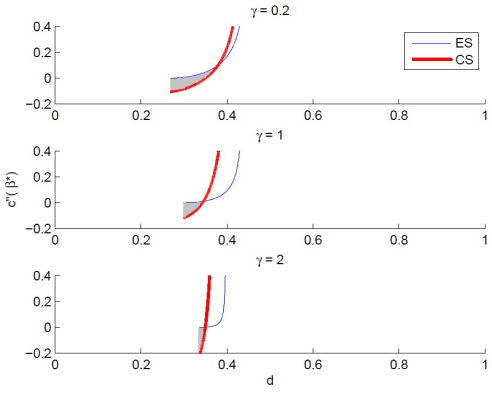


(a) Varying a.

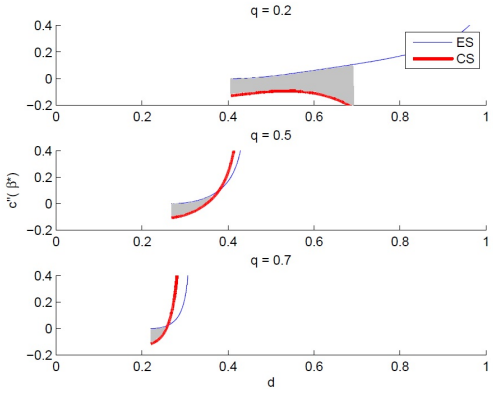
(b) Varying θ.



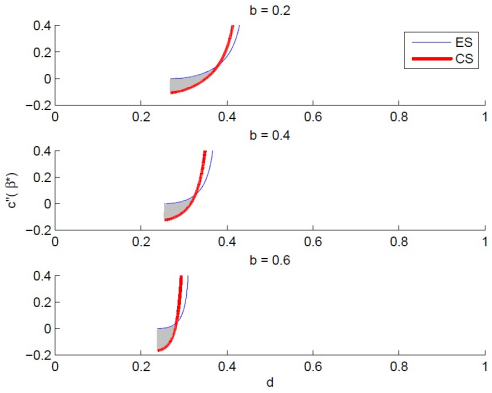
(c) Varying α.



(d) Varying γ.



(e) Varying q.



(f) Varying b.

Figure 3.10: Branching region (shaded region), where $(\beta^*, c^*) = (1.15, 0.08)$. Other parameters are given in Table 3.1.

posing a threat to the susceptible population. This leads to more diversity in the population as some hosts will evolve defences against the disease and others against predation. However, for high ϕ, branching is less likely as the hosts are likely to defend themselves against the predators due to the high risk of predation. In this case, less diversity occurs in the system as the hosts clearly face a high risk of predation making it more beneficial to evolve defences against the predators. Hence, branching is more likely to occur when both the enemy populations are present and relatively balanced posing simultaneous risks of infection and predation, whereas, branching is less likely to occur when one enemy strongly dominates the enemy community composition. I found that similar to ϕ, as a and θ increase, the size of the branching region decreases, whereas, as the parameters α, γ, q and b increase, the size of the branching region increases (shown in Figure 3.10).

3.4 Discussion

I have highlighted the importance of ecological feedbacks to the evolution of host defence within complex communities and shown how these feedbacks drive the evolution of host defence against multiple enemies. In particular, I have shown that investment in defence against parasites and predators varies depending on the composition of the enemy community, as this relates to the relative risk of infection or predation. In general I have found, quite intuitively, that when there is a higher proportion of infected hosts relative to predators, the hosts increase their defences to infection, whereas, when there is a higher proportion of predators, the hosts increase their defences to predation. Thus, the proportion of infected hosts to predators forms a simple ecological driver for the evolutionary behaviour of many host populations. However, I found that this driver is modified to include the relative growth rates of susceptible and infected hosts when there is significant recovery or reproduction from infected hosts ($\gamma > 0$, $f > 0$), highlighting the

important distinction between parasites that do or do not act as 'functional predators' (Boots, 2004). More specifically, given low virulence, our results varied depending on whether the predator selectively targeted susceptible hosts (leading to high defences to predation) or infected hosts (leading to high defences to parasitism). With selective predation of infected hosts, the high risk of predation once infected caused the hosts to switch from defence against predation to defence against infection, despite the predator population being larger than the infected population. Here, alongside the population densities, the relative growth rates of the susceptible and infected hosts also now play a vital role in the fitness term. Hence, given significant recovery and infected host reproduction, the relative abundance of infected hosts to predators is not always the key factor in driving host evolution towards greater defence against the infection or predation. The occurrence of such complicated evolutionary behaviour within the host population shows the importance of wider ecological feedbacks. Although the relative population densities of the enemy populations are important, the evolutionary trends may still be unpredictable due to the complex feedbacks arising between the dynamic population densities. I have also shown that the host population may evolve greater diversity when faced with a combined high risk of infection and predation as the hosts will diversify into specialists with defence mechanisms aimed at either the infection or predation.

It would be easy to dismiss these results as being rather trivial. The fact that host defence is directed against the most threatening enemy, whether that threat is determined solely by relative abundance or in combination with host growth rates, appears straightforward. However, we have in fact uncovered this simple driver as being responsible for the seemingly non-intuitive trends that emerge; trends that can only be understood when we consider these broader ecological feedbacks. For example, we have shown that higher recovery rates from infection lead to increased defence to parasites (if predators selectively prey upon infected hosts); a seemingly counter-intuitive result

as we might presume that high recovery would reduce selection for resistance to disease. However, our analytic evaluation of the fitness gradient shows that in this case the predator density decreases faster than the infected host density, making the infection the bigger threat. Therefore, I emphasise the vital role that ecological feedbacks play in antagonistic evolution within communities.

Resistance against parasitism is often expected to be greatest when the exposure rate to disease is high. A classic result from evolutionary models of host defence to disease is that resistance to sterilising diseases may be lower against highly virulent parasites due to the reduced prevalence of infected hosts (Boots and Haraguchi, 1999). However, here I have found that hosts focus their defences on parasitism rather than predation when virulence is high. This is due to less predators being present in the population when virulence is high, caused by the decrease in the number of infected hosts. This result was found to hold regardless of whether there is selective predation of infected hosts and with the inclusion of an additional trade-off in which there is a cost of high total defence to the host birth rate (see Box 3.1). On the other hand, I found that selection for high defence to predation was greatest when there is strong selective predation of infected hosts and when infected hosts have high fecundity, as both of these processes lead to a larger predator population. I therefore emphasise that the ecological feedbacks generated by both population sizes and relative growth rates are vital to understanding evolution in complex communities.

A crucial question, of course, is whether the predictions from our theoretical model are observed in experimental or empirical studies. One particularly relevant study is that of Friman and Buckling (2012), who not only studied host (bacteria) evolution against two enemies (phage and protists) but also recorded population densities across the evolutionary timescale, a rarity amongst experimental studies. They showed that when *Pseudomonas fluorescens* bacteria were exposed to both enemies, the host population evolved

into two coexisting specialist populations, one with high defence against phage and one with high defence against protists. Similarly, we have predicted that such coexistence can arise through evolutionary branching, specifically when neither of the enemies is dominant (meaning selection is not biased towards a particular enemy), and the trade-off is roughly linear. The population data from Friman and Buckling (2012) appears to agree with this since the densities of the two enemies remained relatively balanced over the evolutionary time period. I would predict that repeating the experiments with one enemy at a higher initial density, either through direct control or manipulation of the environmental conditions, would be more likely to result in a monomorphic specialist or 'biased generalist' dominating in the bacteria with defences aimed at the larger enemy population. Such evolution towards a host with defence mechanisms aimed at the enemy posing a larger threat is evident in other empirical studies.

Using a 50-year long time series on *Perca fluviatilis* perch (host) and *Esox lucius* pike (predator), Edeline et al. (2008) found that without a perch-specific pathogen, the pikes preference for small perch had driven the perch to evolve towards a large body-size. However, after the pathogen was introduced, which is more successful in large perch, selection had driven the perch to evolve towards a small body-size. Not only does this demonstrate an indirect trade-off between defences against different enemies, but it appears that selection depends on the relative threat from each enemy, aligning with our results. Similarly, Craig et al. (2007) showed that evolution in *Eurosta solidaginis* flies (which induce galls on a subspecies of tall goldenrod, *Solidago altissima altissima* and *S. a. gilvocanescens*) is driven by the composition of the enemy community as small galls are more prone to infection and large galls are more likely to suffer predation. Consequently, given a higher chance of infection, the flies evolved towards large galls and given a higher chance of predation, the flies evolved towards small galls. This would be expected from our results as the flies are evolving their defence mechanisms based on

whether there is a higher risk of infection or predation. Although, in regions where there was a combined risk of infection and predation, they found stabilizing selection on gall size, whereas our results would predict disruptive selection to occur where both infection and predation are present. This highlights the more complex behaviours that can arise in nature.

Furthermore, experimental work by Rigby and Jokela (2000) found that as *Lymnaea stagnalis* freshwater snails increased investment in predator avoidance behaviour (as a result of being exposed to the predator more often) they lowered their immune defences against potential pathogens, indicating a trade-off between defences against predators and parasites with evolution seemingly driven by the hosts relative exposure to the two enemies. Another study by Stinchcombe and Rausher (2001) showed that when insects are present, deer are more likely to be attracted to ivyleaf morning glory, causing the plants to evolve higher deer resistance in the presence of insects showing that the plants evolve defences against deer when they are more likely to encounter them.

In a study on a slightly different interaction involving a single enemy and a mutualist population, Siepielski and Benkman (2009) found that when evolving in the presence of both pine squirrels (*Tamiasciurus* spp., seed predators) and Clark's nutcrackers (*Nucifraga columbiana*, seed dispersers), limber pine (*Pinus flexilis*) trees developed more phenotypic variation in their pine cone structure. The limber pine evolved higher defences to squirrels when they were present in the population, simultaneously making their seeds difficult for nutcrackers to disperse so when squirrels were present, the limber pine had to rely on secondary dispersers, highlighting the importance of the surrounding community composition on evolution. These numerous observable traits reveal the crucial effects that community dynamics can have on driving the evolution of host defences in nature.

Additionally, I have shown that more diversity occurs through disruptive selection in populations where the host is likely to have interactions with

both parasites and predators. It is important to note that the type of coexistence I have shown after branching is between hosts whose defence strategies focus on different enemies. This differs from previous theoretical examples of branching in host defence to parasitism where the trade-off is between defence and general life-history traits (Boots and Haraguchi, 1999, Miller et al., 2005, Hoyle et al., 2012 and Chapter 2). I believe that this is the first theoretical study to demonstrate branching to coexistence of this nature. I found that when the hosts face a simultaneous risk of infection and predation then (for certain cost structures) the host population diversifies into coexisting specialists with defence strategies aimed against either parasites or predators. Branching was most likely when selection against one particular enemy was not too strong. For example, diversity was less common when there was high virulence (since this favours high defence to parasitism) or strong selective predation (since this favours high defence to predation). Interestingly, the experimental study of Friman and Buckling (2012) noted that as well as the trade-off between host defence strategies, there appeared to be further costs to life-history traits. I have found here that further costs can still give rise to the coexistence of hosts with varying traits, but we emphasise that these additional costs are not necessary for branching in our model as the trade-off between defence mechanisms alone is sufficient enough to generate the negative frequency-dependence required for coexistence.

The role of community interactions in antagonistic evolution is receiving increasing attention within both mathematical modelling and experimental evolution fields. Clearly, the development of evolutionary theory relies on both of these disciplines working collectively and I believe there are many exciting opportunities for development of this field. Experimental and empirical studies that consider evolution within complex communities must overcome many practical difficulties in maintaining and sampling from such systems. Only a few studies of such evolution against multiple enemies exist (Rigby and Jokela, 2000, Craig et al., 2007, Edeline et al., 2008, Friman and Buck-

ling, 2012). A key insight of our work is that the relative population densities of enemies are a significant determinant of the hosts evolutionary behaviour. While there are undoubtedly practical issues to overcome to record population data during experimental evolution, where this is possible (such as in Friman and Buckling, 2012) this data may provide crucial insights in to the observed traits. Indeed, recording this data may allow for experimental tests of our findings here, specifically to determine how well the relative population densities of two enemies can explain patterns of investment along environmental gradients.

Theoretical studies in this emerging field have investigated both parasite and host evolution in the presence of a (dynamic) immune predator. For parasite evolution, the additional feedbacks from the predator can lead to branching and coexistence of multiple parasite strains (Morozov and Best, 2012), which is prevented due to a competitive exclusion principle when there is no predator (Bremermann and Pickering, 1983, Bremermann and Thieme, 1989; but see Best and Hoyle, 2013 for other counter-examples), as well as cyclic fluctuations of parasite virulence and predator densities (Kisdi et al., 2013). Predation of hosts therefore appears to make diversity of parasites more likely. For the host, it has been shown that defence to parasitism is greatest at intermediate predation rates as this combines high risk and cost of infection (as discussed in Chapter 2), and that hosts can drive their parasite to extinction if the predator is present (Hoyle et al., 2012). There are many potential developments to come in our theoretical understanding in this field. One example is to consider the coevolution of the host population with the enemy populations since the parasite and predator populations may adapt in response to the host. There is much existing work on host-parasite coevolution (van Baalen, 1998, Restif and Koella, 2003, Best et al., 2009, 2010, Boots et al., 2014), for example showing how the degree of static diversity that can arise in hosts and parasites depends on the nature of the infection interaction (Best et al., 2009, 2010, Boots et al., 2014). The work mentioned

above suggests that predation is likely to play an important role here. The existing theoretical work has also focussed on host defence through resistance (specifically avoidance, lowered transmission rate) and it would be interesting to explore how predation might impact the dichotomy between resistance (which causes negative frequency-dependence) and tolerance (which causes positive frequency-dependence; Roy and Kirchner, 2000, Miller et al., 2005, Best et al., 2008). Crucially, I would emphasise that improvements in our understanding of antagonistic evolution in complex communities requires further work both within and between experimental evolution and mathematical modelling.

Chapter 4

The experimental existence of trade-offs between defence against two enemies in *Plodia interpunctella*

4.1 Introduction

In the previous chapters (Chapters 2, 3 and next in Chapter 5), I have studied the theoretical evolution of host-parasite models with the inclusion of a predator population, whilst assuming various trade-offs occurring between traits. In this chapter, I carry out experimental work using *Plodia interpunctella* (Indian meal moth) as the host system to gain experimental evidence of the existence of trade-offs against multiple enemy populations. We considered this scenario in Chapter 3 whilst looking at the evolution of host defences against two enemies, parasites and predators, showing that the relative population densities of the two enemies has an important impact on the evolution of host defence.

In nature, populations typically face multiple enemy populations so they

may have to defend themselves against such populations. For example, red grouse and snowshoe hare populations face risks of infection by parasites along with risks of predation by predators (Hudson et al., 1992, Murray et al., 1997). In response to this, host populations may evolve generalised defence mechanisms which offer protection against multiple enemies. Certain adaptations in the host may reduce encounters with multiple enemies (Paul et al., 2000, Moore, 2002, Poitrineau et al., 2003, Biere et al., 2004). Contrastingly, host populations may face a trade-off between developing defences against enemies as increased defence against one enemy may constrain defence against another. Various experimental studies have also shown evidence of such a trade-off (Rigby and Jokela, 2000, Stinchcombe and Rausher, 2001, Nuismer and Thompson, 2006, Craig et al., 2007, Edeline et al., 2008, Gomez et al., 2009, Siepielski and Benkman, 2009, Friman and Buckling, 2012). Hence, whilst evolving defences against multiple enemies, the host population may evolve generalised defence mechanisms or face a trade-off between defence mechanisms. Given that such diversity can arise in the evolution of host defence mechanisms, I investigate the experimental existence of a trade-off in *P. interpunctella* host defence against two types of infection (i.e. two different enemies), *Plodia interpunctella* granulosis virus (PiGV) and *Staphylococcus aureus* bacteria (*S. aureus*).

Different strains of *P. interpunctella* have been maintained in the lab, specifically, control (non-virus) and virus lines. The virus lines have had prior exposure to PiGV and have been previously assayed showing that they have developed more resistance to PiGV compared to the control lines which have had no prior exposure to PiGV (Boots and Begon, 1993, Tidbury et al., 2010). This links to evidence of immune priming in *P. interpunctella* as early exposure to PiGV increases immune protection to later exposures, however, relatively little is known about the way in which these defence mechanisms work biologically (Tidbury et al., 2010). Additionally, earlier experiments carried out over a two year period by Boots and Begon (1993) also showed

that moths which had been exposed to the virus were more resistant to infection than those from virus-free control populations. These studies clearly show that the virus lines have higher resistance to PiGV in comparison to the control lines. However, this host system has not been tested with *S. aureus* bacteria so it is unknown whether there is a generalised host defence mechanism or a trade-off between defence against these two enemies.

In this experiment, we aim to determine whether the previous exposure of the host to the virus improves or proves costly to their defences against the bacteria. I used *P. interpunctella* that have been selected for resistance against PiGV to see how they respond to a challenge with *S. aureus* to determine whether those that have been selected for resistance to PiGV are more or less resistant to bacteria (or if there is no correlation). The results should reveal the underlying dynamics of any trade-off occurring in the *P. interpunctella* host as they develop resistance against PiGV and *S. aureus*. Specifically, the results should show if the selective pressures imposed upon *P. interpunctella* by virus and bacteria infections lead to a trade-off between defence against the two enemies, such that increased defence against one enemy constrains defence against the other or a generalised defence mechanism providing defences against both enemies. The results could also reveal that there is no correlation between defences against the two enemies (see Section 4.1.1 for the hypotheses).

I begin by testing the resistance of the *P. interpunctella* strains against PiGV and then against *S. aureus*. Given that the *P. interpunctella* host is known to develop resistance to PiGV if it has been previously exposed to the virus, the PiGV experiments should show that the virus lines have higher resistance than the control lines to PiGV. The results from the *S. aureus* experiments will be insightful as the lab has not tested the resistance of the *P. interpunctella* host system to *S. aureus*. I then compare the data from both the virus and bacteria experiments to determine the interaction between any types of defence mechanisms which may occur.

This experimental work was carried out in Professor Mike Boots lab at the University of Exeter, Penryn campus from February to April 2015. The experimental design was created in collaboration with Professor Boots and lab technicians, Steve Sharpe and Toby Doyle. I carried out the experimental work, data collection and analysis.

4.1.1 Hypotheses

The data were analysed to see if larvae treatment lines (i.e. virus and control lines) that were resistant to the virus were more or less resistant to the bacteria or if there was no correlation. See Figure 4.1 for a flowchart of the experimental work. The following are the hypotheses to be tested during the data analysis:

1. Null hypothesis: no correlation of the selection line on the defence mechanisms against the enemy populations.

2. Selection lines more resistant to virus could be more resistant to the bacteria, suggesting that defence mechanisms against the virus are also beneficial to the hosts when defending themselves against bacteria. This suggests that there is a generalised defence mechanism against the two enemies, i.e. no trade-off between defences against the two enemies.

3. Selection lines more resistant to the virus could be less resistant to the bacteria, suggesting a trade-off between defence against the two enemies. In this case, by building up defences against the virus, the hosts constrain the levels of defence that they can build up against the bacteria.

4.2 Methods

Thirty-eight *P. interpunctella* selection lines were used in the experiment, including virus lines (V) which had been previously exposed to virus and thereby selected for resistance against PiGV and control lines (C) which had

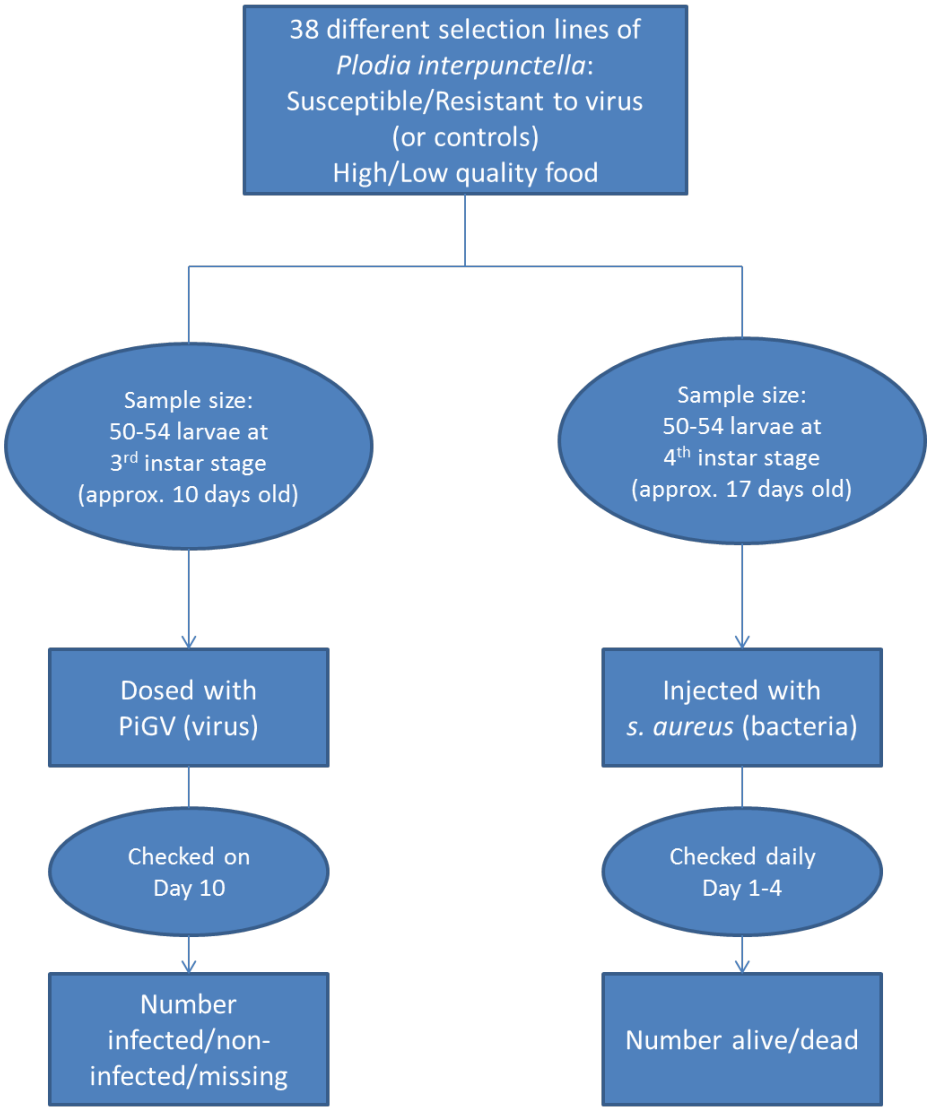


Figure 4.1: Flowchart outlining the experimental work carried out on the *Plodia interpunctella* host system.

not been previously exposed to the virus. These lines were maintained on high or low quality food (HF/LF) (see Box 4.1 for food preparation procedures). This gives the following selection lines which were used in the experiment: VHF, VLF, CHF, CLF. Larvae from each line were dosed with the virus and injected with bacteria. The lethal dosage that kills 50% of the individuals (LD50) for the virus and bacteria were found and used in the experiment (see Boxes 4.2 and 4.3) as this dosage allows for variation in the results. For example, if the LD100 was used, all of the larvae would have been killed. For the virus experiment, viral infection was assayed, measured on day 10, as the food was kept the same, i.e. selection lines raised on HF (or LF) were given HF (or LF) after being infected with virus. For the bacteria experiment, bacterial infection was assayed, measuring over 4 days and again, the food was kept the same.

I look at the effect of selection virus treatment (V/C) and food treatment (HF/LF) and the interaction between the two to determine if there is an effect of selection line on the resistance of the larvae against the virus and bacteria. Intuitively, we would expect higher resistance in the virus treatment lines as these lines have been exposed to the virus and therefore may have developed defence mechanisms against the virus. This has also been shown in previous work done in this lab (Boots and Begon, 1993, Tidbury et al., 2010). There is more uncertainty regarding what would happen in terms of bacterial resistance, as the virus treatment lines may have higher or lower resistance against the bacteria. This has not been previously tested using these selection lines. In terms of food treatment, we would expect the high food lines to have greater resistance against virus and bacterial infections as they are given a better resource so they should have more to spend on building up defence mechanisms (Boots, 2011). However, the experiments may show that there is no effect of selection line on the defence levels.

Figure 4.2: *Plodia interpunctella* larvae and adult moth (Rees, 2007).

4.2.1 *Plodia interpunctella* host system

For this experiment, *P. interpunctella* (shown in Figure 4.2) was used as the host system as they are relatively easy to maintain in a lab environment. They have a short life-cycle of approximately 30 days which makes it easy to look at the existence of any trade-offs occurring between defence mechanisms during their life span. Their eggs grow in their food source and the larvae are external feeders which produce silk webbing (Rees, 2007). After pupation, they reach a short adult moth stage in which they do not consume any food (Rees, 2007). Additionally, as the lab has previously exposed some of the strains to PiGV creating populations which have been selected for resistance against the virus, this made them ideal for the experiment.

4.2.2 *Plodia interpunctella* granulosis virus and *Staphylococcus aureus* bacteria

PiGV and *S. aureus* were used as the two infections for the *P. interpunctella* host system in this experiment. PiGV is a naturally occurring DNA virus which infects larvae once virus occlusion bodies have been ingested and dissolved in the midgut prior to entering the midgut epithelium cells. Virus proliferation occurs, causing cell lysis and tissue destruction which eventually leads to the death of the host. Additionally, as the host dies, their

hypodermis ruptures, thereby releasing occlusion bodies which may then be ingested by other hosts (Boots and Begon, 1993, Tidbury et al., 2010). We can determine visually which larvae have been infected with PiGV as they turn opaque white and do not reach the pupation stage (Boots and Begon, 1995).

Although I use the bacterial pathogen, *S. aureus*, to infect the *P. interpunctella* larvae, it is also capable of infecting humans, mostly through community and hospital-acquired infections (Garcia-Lara et al., 2005). Pathogenesis by the bacteria can cause a variety of diseases as it is able to effect various systems and organs (Garcia-Lara et al., 2005). An innate immune response is typically initiated in response to a bacterial infection (Garcia-Lara et al., 2005). In order to infect *P. interpunctella* with the virus, it is fed to them in their food source and to infect with the bacteria, it is injected into the larvae.

4.2.3 Dosing procedure

Fifty larvae at the third instar stage (approximately 10 days old) from each selection line were dosed with the LD50 virus (see Box 4.2 for obtaining the LD50 for the virus), which was given in their food by pipetting drops of virus into their petri dish. Coomassie brilliant blue dye was added to the virus before dosing to ensure visually that it had been consumed by the larvae (see Figure 4.3). After approximately 30 minutes, 25 larvae were removed from the petri dish and placed into a well (see Figure 4.4) which contained their food (LF/HF corresponding to which selection line they were from). They were then kept in an incubator at 27°C. Some petri dishes were placed in the freezer to check later which does not affect the results. Here I am only assaying treatment line (V/C) whilst checking the number of infected (white, non-clear larvae), non-infected (peachy, some colour in larvae) and missing (larvae not found visually) larvae 10 days later. For the dosing procedure, third instar larvae were used as they need to be left for 10 days to allow the

Box 4.1: Food preparation

To set up the pots: 200g of food were added into each pot; 10 moths from 5 different pots giving 50 moths in each pot were placed in with the food. A net was placed on top to stop the moths from getting out via holes in the pot lid. The following food preparation procedures are from Professor Mike Boots' lab group.

Cereal mix and normal food

For the cereal mix use the following: 500g ready brek, 300g bran and 200g ground rice.

For the normal food use the following: 400g cereal mix, 80g Brewers' yeast, 1.7g methyl paraben, 1.7g sorbic acid, 100ml glycerol and 100ml honey.
The dry ingredients were mixed with the glycerol and honey for 10 minutes using a mixer set on level 1.

High Quality Food (HF)

For the HF use the following: 450g cereal mix, 50g methyl cellulose, 100g Brewers' yeast, 2.1g sorbic acid, 2.1g methyl paraben, 116ml glycerol and 116ml honey. The same mixing procedure as stated for the normal food was used.

Low Quality Food (LF)

For the LF use the following: 225g cereal mix, 275g methyl cellulose, 100g Brewers' yeast, 2.1g sorbic acid, 2.1g methyl paraben, 116ml glycerol and 116ml honey. The same mixing procedure as stated for the normal food was used.

Figure 4.3: Food preparation in the lab.

The addition of more methyl cellulose reduces the resource quality of the food (Boots and Begon, 1995).

Figure 4.3: Larvae after dosing procedure, the blue larvae has ingested some of the virus (image on the left) and the white larvae is infected with the virus (image on the right). The other larvae is healthy and non-infected.

virus infection to develop and infect the larvae; additionally, they need to be checked before they begin to pupate (pupation occurs the fourth instar stage) so they are dosed at this early instar stage.

Note regarding the cleaning procedure: surface areas were cleaned with ethanol and the UV box was used for virus work.

4.2.4 Injecting procedure

One week later, 54 larvae at the fourth instar stage (approximately 17 days old) from each selection line were injected with LD50 bacteria (see Boxes 4.3 and 4.4 for obtaining the LD50 for the bacteria and growing up procedure). Fourth instar larvae were used for the injecting procedure as third instar larvae were too small to inject. Coomassie brilliant blue dye was also added to the bacteria before injecting it to ensure visually that it had been injected into the larvae successfully as they turn slightly blue once injected. Whilst injecting, the larvae were turned upside down on a petri dish and kept in place with cling film to restrict their movement. Additionally, placing the larvae on ice lowered their movement. A picopump was used to inject the larvae in their third proleg using a capillary needle (see Box 4.5 for the needle pulling procedure). The injection eject pressure was set to approximately 14 psi and range 100s – period 20ms on the picopump. After being injected, the

Box 4.2: LD50 for virus

The sonocator was used to break up any of the stock virus stuck together. 100 larvae were dosed with the virus (different virulence levels and a control sample dosed with sugar instead of virus, see Table 4.1 for the virus doses) and placed in a petri dish. After approximately 30 minutes, 50 larvae which had been infected with the virus were selected (larvae turn slightly blue once they have consumed some of the virus). The larvae were then placed into square petri dishes with low or high quality food (same as the food they had been maintained on) and placed in an incubator at 27°C. 10 days later, the number alive, dead and missing was checked (see Table 4.2 for the data gathered). Figure 4.4 shows that virus dose 2 has the closest virus concentration that infects approximately 50% population so this was used as the LD50 virus. More of the LD50 virus was produced and frozen for when needed. Before using the virus for the experimental procedure, it was left to melt and vortexed.

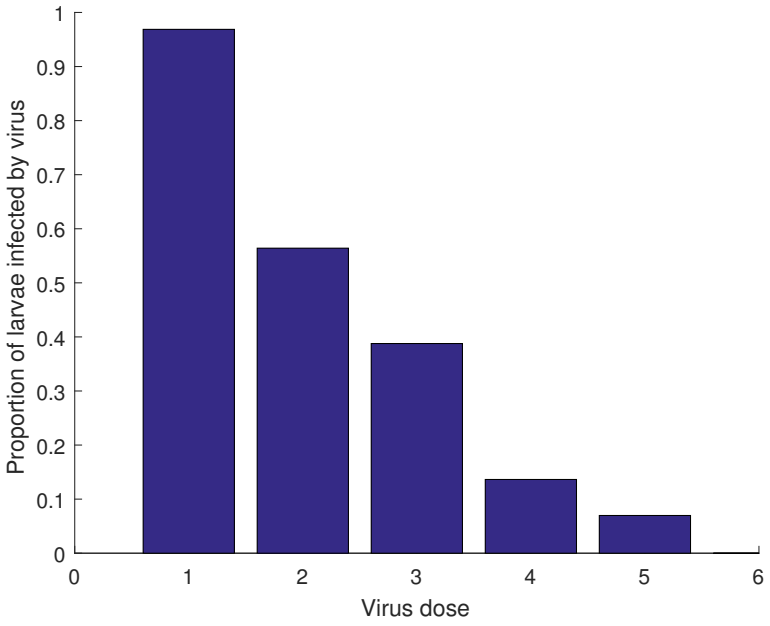


Figure 4.4: LD50 virus results showing that virus dose 2 is closest to an LD50.

Figure 4.4: Larvae dosed with virus placed in a 5×5 square petri dish (one larvae placed in each well).

Table 4.1: Virus doses

Virus Dose	Concentration	Mixture
Stock	2.4×10^{-3}	start 1:10 for 1 and 2 then 1:5 ratio
1	2.4×10^{-4}	3ml stock + 27ml H_2O
2	2.4×10^{-5}	3ml 1 + 27ml H_2O
3	1.375×10^{-5}	6ml 2 + 24ml H_2O
4	2.4×10^{-6}	6ml 3 + 24ml H_2O
5	1.375×10^{-6}	6ml 4 + 24ml H_2O
6	2.4×10^{-7}	6ml 5 + 24ml H_2O
CS	0	Control sucrose (mixture=1g dye+ 4g sucrose, 47.5ml H_2O+2.5ml mixture)

Table 4.2: Results for LD50 virus tests

Virus dose	Number non-infected	Number infected	Proportion infected
1	1	31	0.9688
2	17	22	0.5641
3	30	19	0.3878
4	38	6	0.1364
5	40	3	0.0698
6	46	0	0

Figure 4.5: Larvae being selected for dosing and injecting procedures.

larvae were placed in containers with their food (LF/HF corresponding to which selection line they were from) and kept in an incubator at 27°C. Here I am only assaying treatment line (V/C) whilst checking the number of larvae alive and dead over 4 days. For this procedure, the larvae were checked daily and a slightly larger sample size (relative to the dosing procedure, i.e. 54 rather than 50 larvae) was used to gain a clear and more accurate insight into how they were affected by the bacteria as they have not been tested with the bacteria before. The bacteria infection seemed to act faster than the virus infection so the larvae were checked daily over 4 days, rather than on day 10. The same larvae populations were not used for the dosing and injecting procedures as it would have been difficult to determine whether the larvae had died from the virus or bacteria.

Note regarding the cleaning procedure: surface areas were cleaned with bleach followed by ethanol and the hood or a bunsen burner were used for bacteria work.

Box 4.3: LD50 for bacteria

10 larvae were injected with each different bacterial concentration and a control sample was injected with PBS alone (see Table 4.3 for the bacteria doses). The larvae were checked daily for 10 days to see the proportion infected. The data showed that bacteria dose 3 had the closest bacteria concentration that infected approximately 50% population (by day 5) so this was used as the LD50 bacteria.

To get concentration of the LD50 bacteria:
$100\mu l$ (0.1ml) sterile water and 1 pump of LD50 bacteria were placed on an agar plate. This was repeated on 6 plates and incubated overnight at 37°C. A plate reader was then used to count the number of colonies.

Table 4.3: Bacteria doses

Bacteria dose	Concentration	Mixture
Control		$100\mu l$ PBS
1	1:1	$100\mu l$ PBS + $100\mu l$ bac (pure=pellet + $500\mu l$ PBS)
2	1:10	$100\mu l$ PBS + $100\mu l$ 1
3	1:100	$100\mu l$ PBS + $100\mu l$ 2
4	1:1000	$100\mu l$ PBS + $100\mu l$ 3
5	1:10000	$100\mu l$ PBS + $100\mu l$ 4
6	1:100000	$100\mu l$ PBS + $100\mu l$ 5
1a	1:5	$50\mu l$ PBS + $100\mu l$ bac (pure)
2a	1:50	$50\mu l$ PBS + $100\mu l$ 1a
3a	1:500	$50\mu l$ PBS + $100\mu l$ 2a
4a	1:5000	$50\mu l$ PBS + $100\mu l$ 3a
5a	1:50000	$50\mu l$ PBS + $100\mu l$ 4a

Box 4.4: Growing up bacteria

The original *S. aureus* bacteria came from a freezer stock. The top of the stock was placed in 500μl of LB broth and incubated over night at 37°C. The next day it was centrifuged at 13kRPm for 4 minutes. The supernatant was discarded (leaving the pellet) before adding 500μl of PBS into the tube. The solution was pipetted up and down to re-suspend bacteria. This was then placed in the fridge to be used for up to 1 week.

A bunsen burner was used whilst isolating a colony. Different bacterial dilutions (1:1, 1:10, 1:100, 1:1000) were produced using sterile water for the dilution to get more spread out colonies. 100μl of each dilution were added to an agar plate and then incubated over night at 37°C. The next day, an isolated colony was found (here, the 1:1000 bacterial dilution was used) and added to 1ml LB broth. Incubated over night at 37°C (on the shaker), which produced fridge (used for up to 1 week) and freezer stock next day. The freezer stock was stored in a labelled cryogenic vial in the –80°C freezer.

Box 4.5: Needle pulling

The needles must be long, sharp and small enough to inject larvae at the fourth instar stage. The following settings were used on the needle puller for orange needles (10μl):

Heat=100 (max), Mag sub=61 (max), Mag main=50 (min)

This produced very long straight needles (the needles can snap and cannot be used for injecting once the tips break off).

4.3 Data analysis

I begin by analysing the data from the virus and bacteria experiments separately and then compare them. The data from the virus experiment should align with previous work clearly showing that the virus lines have higher resistance than the control lines (Boots and Begon, 1993, Tidbury et al., 2010). However, the data from this experiment do not show this result so given this discrepancy, we cannot assume that the data from this experiment can be used to make any reliable conclusions about the *P. interpunctella* host system. Regardless of this, an analysis of the gathered data from the experiment was carried out whilst noting that there may be errors with the data. Repeating the experiment with improvements would potentially allow us to determine the existence of any defence trade-offs against the two enemies, virus and bacteria, for the *P. interpunctella* host.

In the following analysis, a general linear model (glm) was fit to the data using R to determine the relationship between the variables, for example, the number of larvae infected and selection line. I used the glm to check whether the proportion of larvae infected with virus or bacteria depended on the selection line (C/V, HF/LF and both together CHF/CLF/VHF/VLF). The p-values ($\Pr(> |z|)$ value from the R glm fit) are used to determine whether the result is significant or not, where a p-value greater than 0.05 is non-significant. The p-values are given in the figure captions.

4.3.1 Virus data analysis

The virus data consisted of selection lines (C/V and LF/HF), number infected, number non-infected, number missing on day 10. Sample size used: 20 control lines (11 LF, 9 HF), 18 virus lines (10 LF, 8 HF). 50 larvae were dosed with the virus from each line. The data was analysed to see if C/V treatment lines or LF/HF types were more resistant to virus infection (and any interaction between the two).

The virus data did not show a significant difference such that the V lines had a lower proportion of infected larvae, compared to the C lines, as is known for this system from previous work. Looking at the data shown in Figure 4.7a, we see that the V lines had a non-significantly higher proportion of infected larvae. We would expect the V lines to have built up more resistance to the virus. Since this known result is not shown by the data, we cannot assume that any of the data from this experiment can be used without repeating the experiment again to ensure that no errors were made. In terms of a difference between the number of larvae infected based on their food type, Figure 4.7b shows that the LF lines had a significantly higher proportion of infected larvae, compared to the HF lines. We would expect this since the HF lines have more resources to spend on building up defence mechanisms and this aligns with previous work. However, given the prior mentioned discrepancy in this data, these results should be treated with caution. Here I have completed the data analysis to get a thorough picture of the results available.

Partitioning this data into subsets, Figure 4.8 shows that only the HF types give the unexpected result of the V lines being less resistant than the C lines (significant p-value). Hence, there may have been an error with the experimental procedure for the HF types. For the LF types, the result was non-significant showing that the C lines were less resistant than the V lines which makes sense intuitively. For C lines, the HF types are significantly more resistant but for the V lines, the HF type are non-significantly less resistant.

4.3.2 Bacteria data analysis

The bacteria data consisted of selection lines (C/V and LF/HF), number alive, number dead on day 1, 2, 3 and 4. Sample size used: 19 control lines (11 LF, 8 HF), 17 virus lines (10 LF, 7 HF). 2 selection lines were excluded from the bacteria data analysis due to incomplete data. 54 larvae

were injected with the bacteria from each line. The data was analysed to see if C/V treatment lines or LF/HF types were more resistant to bacterial infection (and any interaction between the two). I exclude deaths that occur on day 1 as I assume that these are due to the injecting procedure and not the bacteria infection.

Figure 4.9a shows that the V lines had a non-significantly higher proportion of infected larvae, compared to the C lines, so these results do not reveal which are more likely to be infected. In terms of looking at the impact of the food type, Figure 4.9b shows that the LF lines had a higher proportion of infected larvae, compared to the HF lines making sense intuitively (significant p-value on days 2 and 3 but not significant p-value on day 4). By day 4, the proportion of larvae infected with bacteria for the LF and HF lines balances out making the difference no longer significant. Again, as many of the results are non-significant we cannot make any definitive conclusions using this data.

After splitting this data into the four different subsets depending on the line (C/V) and food type (HF/LF), Figure 4.10 shows the proportion of these infected with bacteria over day 2 to 4. It shows that only the HF types give the result of the V lines being less resistant than the C lines, i.e. the VHF lines have a higher proportion of infected larvae compared to the CHF lines from day 2 to 4 (significant p-value; as was also found for the virus data). For the LF types, the result was non-significant showing that the C lines were less resistant than the V lines, i.e. the CLF lines have a higher proportion of infected larvae compared to the VLF lines from day 2 to 4 (also found for the virus data). For the V lines, the HF types are more resistant than the LF types, i.e. the VLF lines have a higher proportion of infected larvae compared to the VHF lines from day 2 to 3, but not on day 4 (this was only significant using the p-value on day 4). For the C lines, the LF lines are less resistant than the HF lines, i.e. the CLF lines have a higher proportion of infected larvae compared to the CHF lines from day 2 to 4 (significant

p-value; also found for the virus data).

4.4 Virus and bacteria data comparison

Next, I compare the data from both the virus and bacteria experiments to determine the type of trade-off occurring between resistance against these two enemies for the *P. interpunctella* host system. In summary, the virus data showed that the C lines had non-significantly higher resistance to the virus compared to the V lines and the HF lines had significantly higher resistance to the virus compared to the LF lines. This is similar for the bacteria data suggesting that HF lines which are more resistant to the virus and also more resistant to the bacteria. In terms of the C and V lines, the non-significance of the data does not allow us to make any conclusions. Therefore, our data does not reveal the underlying defence mechanisms at play.

Further splitting the data into subsets, both the virus and bacteria data show that the V lines were significantly less resistant for the HF data only (for LF data, V lines were non-significantly more resistant). The virus data shows that the LF lines were non-significantly less resistant for the C data only (for V data, HF lines were non-significantly less resistant). The bacteria data also shows that the LF selection lines were non-significantly less resistant for the C data, as well as the V data (except on day 4 where V data shows that HF lines were significantly less resistant). The results are summarised in flowchart 4.11. Overall, given the discrepancies and non-significance of the results, we cannot determine the *P. interpunctella* defence mechanisms acting against the virus and bacterial infections without repeating the experiment.

4.4.1 Summary of hypotheses

Analysis of the data was carried out to see if larvae treatment lines (i.e. virus and control lines) that were resistant to the virus were more or less resistant to the bacteria or if there was no correlation. The p-values from the glm test

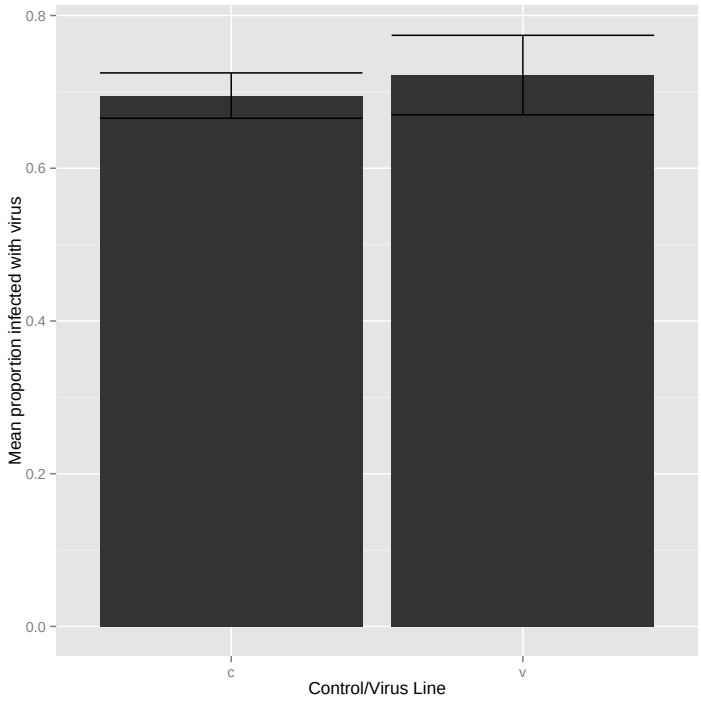
in R were used to determine the significance of the results, where a p-value greater than 0.05 is non-significant. The following hypotheses were tested during the data analysis:

1. Null hypothesis: no correlation of the selection line on the defence mechanisms against the enemy populations.

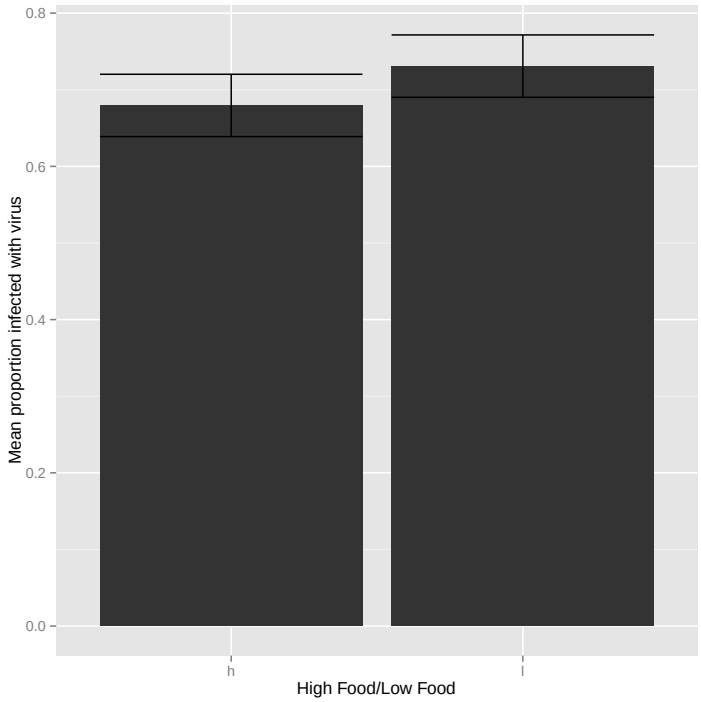
2. Selection lines more resistant to virus could be more resistant to the bacteria, suggesting that defence mechanisms against the virus are also beneficial to the hosts when defending themselves against bacteria. This suggests that there is a generalised defence mechanism against the two enemies, i.e. no trade-off between defences against the two enemies.

3. Selection lines more resistant to the virus could be less resistant to the bacteria, suggesting a trade-off between defence against the two enemies. In this case, by building up defences against the virus, the hosts constrain the levels of defence that they can build up against the bacteria.

Given discrepancies in the data analysis and the non-significance of the results, the null hypothesis cannot be rejected.



(a) Different selection lines, p-value = 0.135 (not significant). Mean proportion of missing larvae is 0.21 for the C and V lines.



(b) Different food types, p-value = 0.0162 (significant). Mean proportion of missing larvae is 0.17 and 0.24 for the HF and LF lines, respectively.

Figure 4.7: Mean proportion of larvae infected with virus.

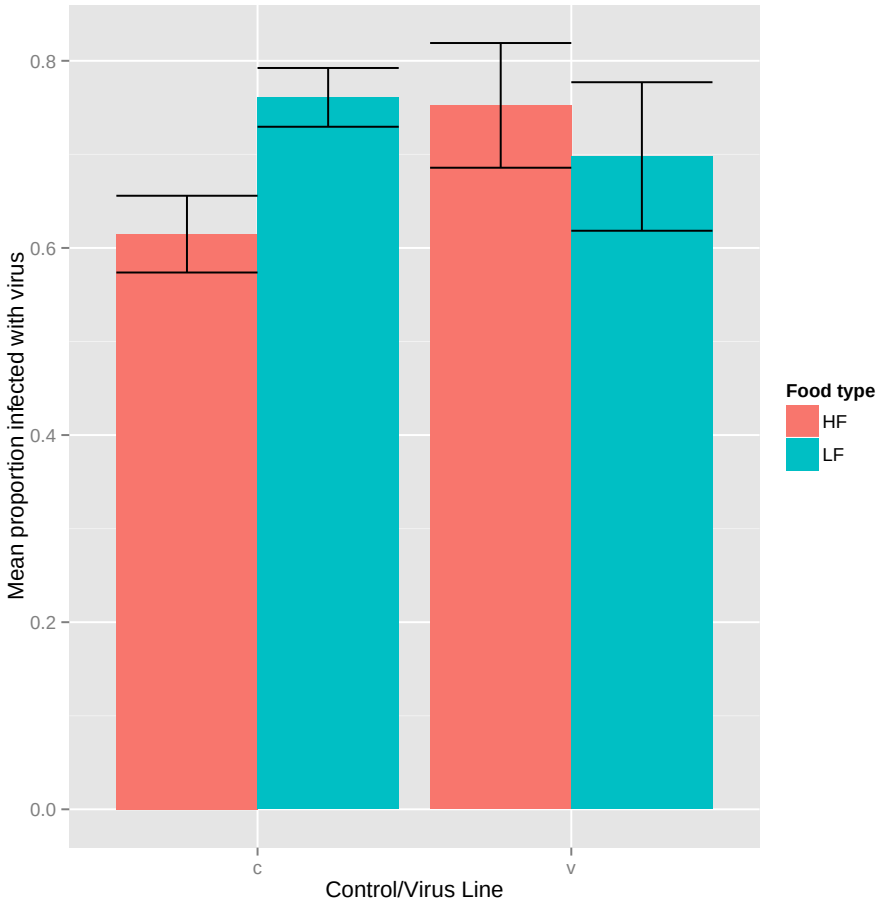
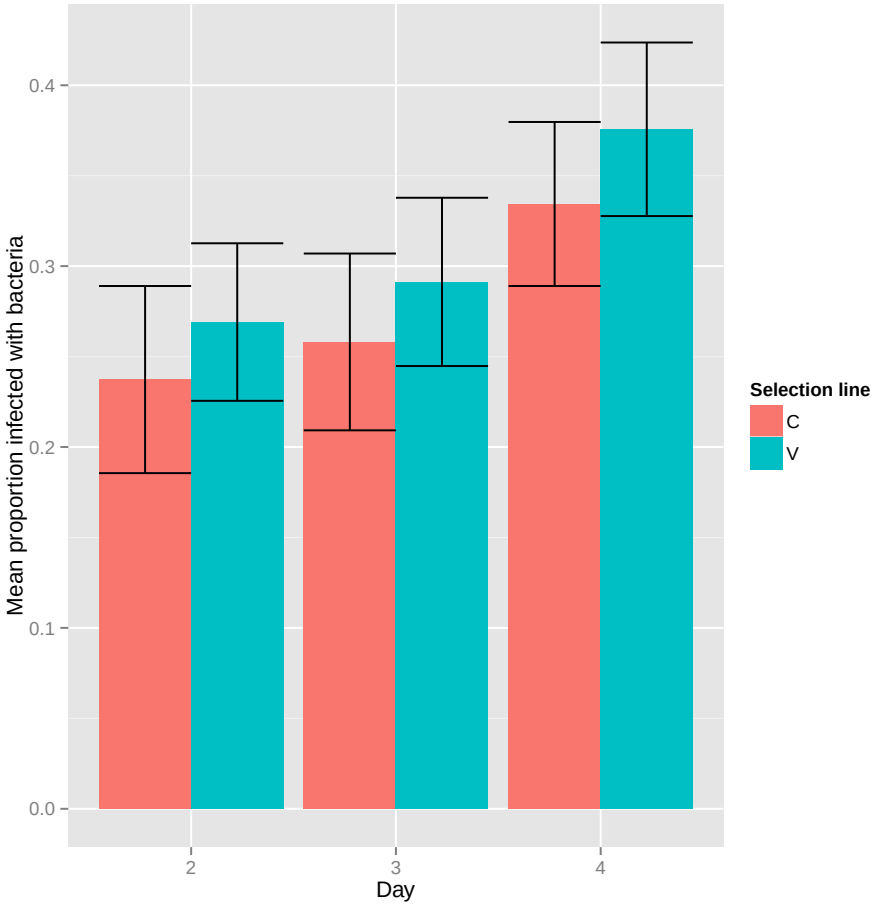
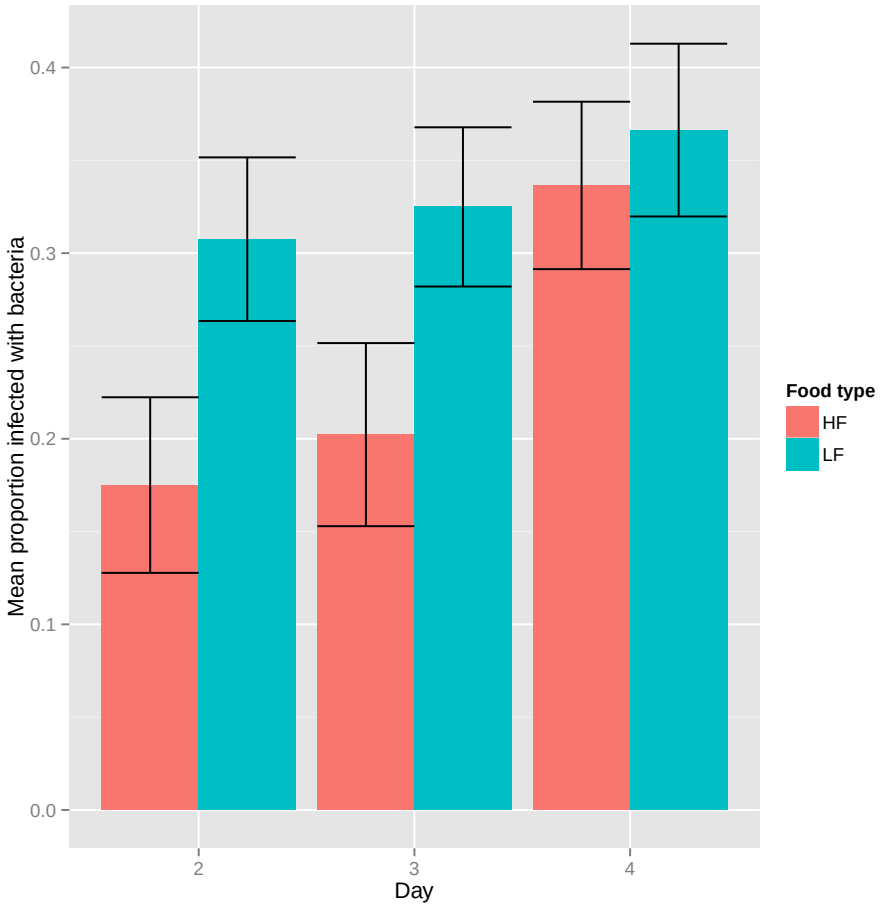


Figure 4.8: Mean proportion of larvae infected with virus depending on selection line and food type, p-values: For C, LF/HF 1.61e-05 (significant), for V, LF/HF 0.268 (not significant), for LF, C/V 0.122 (not significant), for HF, C/V 0.000195 (significant). Mean proportion of missing larvae is 0.19, 0.23 0.15 and 0.26 for the CHF, CLF, VHF and VLF lines, respectively.



(a) Different selection lines, p-values: For day 2 0.263, for day 3 0.256 and for day 4 0.304 (not significant).



(b) Different food types, p-values: For day 2 0.00334 (significant), for day 3 0.00812 (significant) and for day 4 0.754 (not significant).

Figure 4.9: Mean proportion of larvae infected with bacteria. Excluding deaths that occur on day 1.

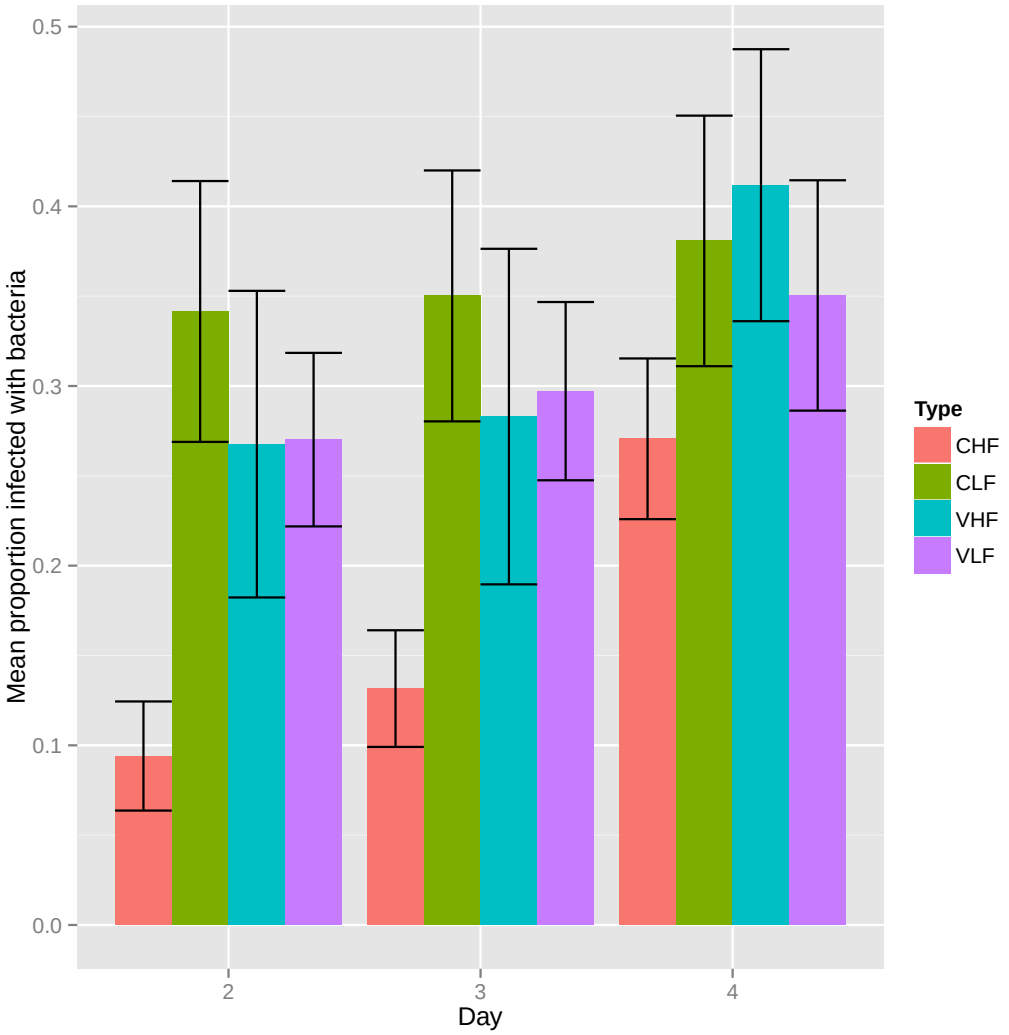


Figure 4.10: Mean proportion of larvae infected with bacteria depending on selection line and food type, p-values: Day 2- For C, LF/HF 1.57e-07 (significant), for V, LF/HF 0.241 (not significant), for LF, C/V 0.0562 (not significant), for HF, C/V 4.81e-06 (significant). Day 3- For C, LF/HF 1.54e-06 (significant), for V, LF/HF 0.296 (not significant), for LF, C/V 0.103 (not significant), for HF, C/V 2.77e-05 (significant). Day 4- For C, LF/HF 0.0497 (significant), for V, LF/HF 0.0211 (significant), for LF, C/V 0.245 (not significant), for HF, C/V 0.00319 (significant). Excluding deaths that occur on day 1.

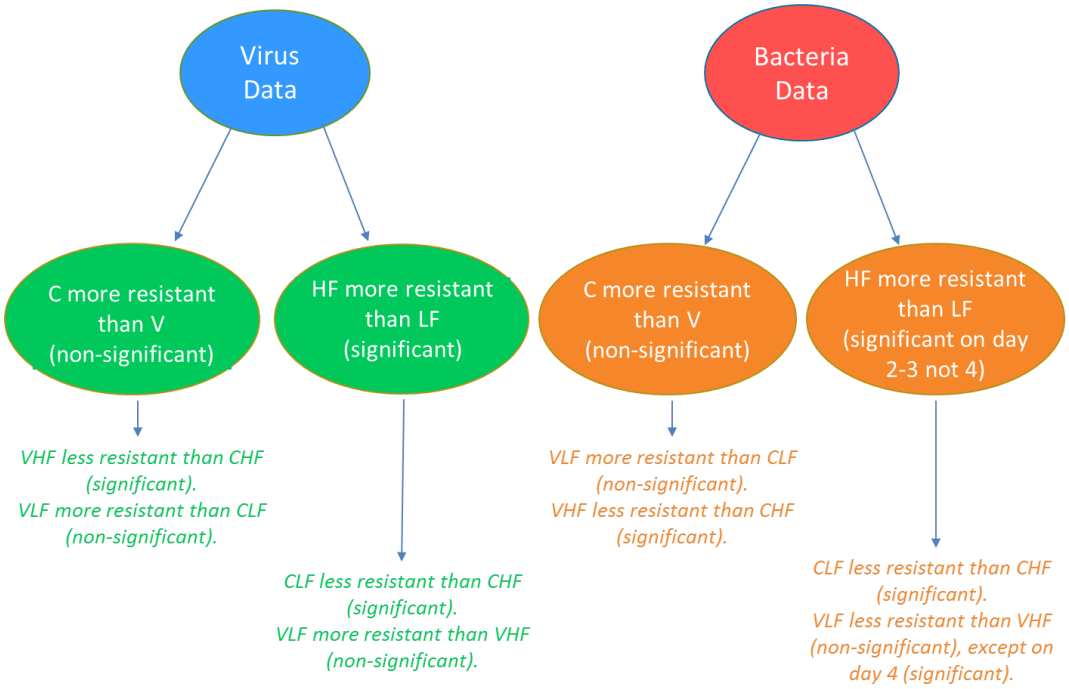


Figure 4.11: Summary of lab results.

4.5 Discussion

The experiment tested whether *P. interpunctella* which had developed resistance to granulosis virus were more or less resistant to bacteria, or whether there is no interaction between defences against these two enemies. As the data from the experiment did not show a significant difference such that the virus lines had higher resistance than the control lines to their granulosis virus (which is known for this system from previous work), we cannot use the data from this experiment to determine the evolution of defences in *P. interpunctella*. In terms of the bacteria resistance experiment, we cannot confirm whether the results are reliable as the lab has not previously tested their *P. interpunctella* lines to check resistance against this bacteria. Here, we discuss the results shown by analysis of the data from this experiment, although it is important to note that we cannot make any explicit inferences about the *P. interpunctella* system.

By infecting the *P. interpunctella* host with PiGV virus and *S. aureus* bacteria, we aimed to determine whether there is evidence of a generalised defence mechanism or a trade-off between developing defences acting against these enemies. Generalised defence mechanisms have been found in other systems as certain adaptations to one enemy have been shown to also confer an advantage against others, for example, a behavioural change may reduce encounters with multiple enemies (Moore, 2002). More specifically, a study by Biere et al. (2004) on the ribwort plantain, *Plantago lanceolata*, showed that plants selected for high-leaf iridoid glycoside concentrations had higher resistance to an insect herbivore (*Spodoptera exigua*) and a fungal pathogen (*Diaporthe adunca*). Thus suggesting that iridoid glycosides provide a generalised defence against these two enemies. Various plant responses to multiple enemies are also discussed by Paul et al. (2000). Furthermore, a theoretical study by Poitrineau et al. (2003) showed that although it is generally expected that a high encounter rate with a certain enemy may reduce investment in defence against another enemy, in some cases it may also improve

resistance against another enemy depending on the mechanistic interactions occurring between the defences. Contrastingly, other biological populations have shown that there exists a trade-off in defence against two enemy populations, such that increased defence against one enemy will constrain defence against another. For example, Friman and Buckling (2012) showed this for bacteria developing resistance against phage and protists (refer to Chapter 3 for more on this type of trade-off with specific examples also shown by Rigby and Jokela, 2000, Stinchcombe and Rausher, 2001, Nuismer and Thompson, 2006, Craig et al., 2007, Edeline et al., 2008, Gomez et al., 2009, Siepielski and Benkman, 2009). This work shows the diversity in the ways in which defence mechanisms can be developed by host systems.

In this experiment, I aimed to determine whether there are any trade-offs between *P. interpunctella* defence against multiple enemy populations. However, the build up of defences against PiGV may also be costly to the life-history traits of the *P. interpunctella* host, similar to Chapter 2 where we discussed a lowered birth rate as host investment in defence increased. More specifically to the *P. interpunctella* host system, Boots and Begon (1995) showed that an increased defence response to granulosis virus may be costly to fecundity, pupal size and mortality at adult emergence. Additionally, Boots and Begon (1993) showed that strains of *P. interpunctella* previously exposed to the virus are able to develop increased resistance but this leads to a longer larval development time and lower egg viability. These studies reveal the deeper complexities that may be arising within this host system as it develops resistance against PiGV. As the evolution of *P. interpunctella* defences against *S. aureus* have not been tested previously, the costs to developing defence against this bacteria remain unclear.

The results showed a significant difference between the resistance developed by *P. interpunctella* depending on the food source that they had been maintained on. Though we note that the food source was not assayed, i.e. larvae raised on low quality or high quality food were given the same food

after dosing or injecting. The results showed that the high food lines had developed more resistance to PiGV, compared to the low food lines, aligning with previous work done by Boots (2000) which showed that *P. interpunctella* given low quality food were more likely to be infected by granulosis virus. The results also showed that the *P. interpunctella* maintained on low quality food had higher rates of infection by the bacteria, compared to those fed on high food. Further work by Boots (2011) has shown that in high-resource environments, *P. interpunctella* are able to develop more resistance and given low-resource environments, the costs of developing resistance are higher so less resistance is evolved. High food may potentially mask costs to other traits since the hosts can use the additional resources from the food towards these traits. Overall, as we would expect intuitively, the results showed that larvae raised on high quality food had better defences against both the virus and bacteria compared to those maintained on low quality food.

Although previous experimental studies have shown the possible existence of generalised defence mechanisms (Paul et al., 2000, Moore, 2002, Poitrineau et al., 2003, Biere et al., 2004), it would be insightful to carry out further experiments on the *P. interpunctella* system to determine whether there is a generalised defence mechanism or trade-off between defences as they defend themselves against the virus and bacteria. Using our results, the defence mechanisms remain unclear for this system. There was no significant difference from the virus experiment data to show clearly that the virus lines had higher resistance than the control lines. Hence, it is possible that an experimental error may have occurred or that there may have been an error in the data collection, as it may not have been correctly noted or the larvae may have been counted incorrectly. The experimental procedure could be repeated with improvements in order to gain clearer results. For example, the larvae selected could be checked carefully to ensure that they have ingested a sufficient amount of virus during the dosing procedure. The LD50 virus and bacteria produced could be tested to make sure that they are of the correct

concentration, as if they are too weak, they would not be strong enough to actually infect the larvae. Also, a larger sample size of larvae could be used when repeating the experiment to gain a more accurate result. As part of the bacteria injecting procedure, a control experiment could be carried out by injecting some larvae with a harmless substance, to ensure that the deaths are due to the bacteria and not the injecting procedure itself. It would be beneficial to find an alternative method of infecting the larvae with the bacteria as the injecting procedure is quite time-consuming, given the quantity of larvae which need to be injected. Lastly, in terms of the food quality, the food type could also be assayed to clearly determine the effect of varying food quality or to simplify the experiment, the larvae could all be maintained on the same type of food.

Conducting experiments can be difficult as we have shown that reliable data giving significant results may not always be produced and this could be due to various errors. Overall, due to discrepancies in the data we cannot determine the resistance of the *P. interpunctella* system without repeating the experiment, or improving the experimental procedure, in order to get a better insight into the evolution of resistance against the virus and bacteria. In Chapter 3, I assumed a trade-off in host defence against two enemies and the results from this mathematical model showed interestingly that the surrounding population dynamics play a key role in the evolution of the host. It would be insightful to see how such results can be applied to host systems in nature by carrying out further work in this area. In the future, continued integration of theoretical work with experiments could be done to see if there is evidence of defence trade-offs occurring in real host systems. This is a field in which more insight is needed and it will be interesting to see how our knowledge in this area develops as we continue to gain a deeper understanding of host defence mechanisms.

Chapter 5

The evolution of host manipulation by parasites

5.1 Introduction

In Chapters 2 and 3, we have focussed on the evolution of the host population in the presence of a parasite and predator population, however, it is also important to consider the evolution of the parasite population to fully understand the behaviour of these systems. Parasites are ubiquitous in nature and are able to adopt a range of strategies in order to complete their life-cycle. For example, parasite transmission can be through direct or sexual contacts; they can be carried by vectors or have free-living environmental stages (Day, 2001, Taylor et al., 2001, Pietrock and Marcogliese, 2003, Shelton, 2004). Many parasites have a two-stage transmission process in which an intermediate host must first be infected, with the disease then passed on to the final host through predation. An intriguing possibility is that many of these parasites are able to manipulate the behaviour of their intermediate host making them more conspicuous to predators (Moore, 2002). While there is much empirical evidence of this behaviour, suggesting that manipulation is most likely to evolve when the predator is the final host for the parasite, becom-

ing infected after preying upon an infected prey (House et al., 2011, Maure et al., 2013, Mitra et al., 2013, Weinersmith and Faulkes, 2014), further work is needed to improve our theoretical understanding of the conditions under which such manipulative parasites could evolve.

Manipulation is not observed in all parasite species that have intermediate hosts, indicating that there may be costs associated with evolving such behaviour. Without any costs, we would expect all parasites to evolve maximum levels of manipulation in order to maximise their rate of transmission to their final host. Although parasites benefit from increased transmission when they raise their levels of intermediate host manipulation (Poulin, 2010), increasing manipulation has been shown experimentally to be costly to the parasites life history traits, such as parasite growth and spore production rates (Poulin, 1994, Vizoso and Ebert, 2005, Frost et al., 2008, Franceschi et al., 2010, Cressler et al., 2014). There may also be consequential costs of manipulation as intermediate hosts could be consumed by an unsuitable predator. Additionally, energy invested in host manipulation will not be available for growth, reproduction or fighting the hosts immune system. Considering that there may be such physiological costs or a higher probability of early death in order to manipulate hosts, Poulin (1994, 2010) suggested that the parasites' investment in manipulation should evolve towards an optimal value which maximises its fitness. These studies show evidence of costly parasite manipulation, however, it has not been shown theoretically when the parasites will invest in manipulation rather than their life history traits, such as spore production.

Previous work on parasite evolution has generally focussed on single host-single parasite systems whilst excluding other population interactions. Recently, theoretical studies have begun to consider the evolution of parasite virulence with the inclusion of a predator population showing that this can lead to the evolution of highly virulent parasites consequently driving the predator population down to extinction (Morozov and Adamson, 2011). Par-

asite diversity occurring through evolutionary branching can increase leading to coexisting parasites with varying virulence levels (Morozov and Best, 2012) rather than classic R_0 maximisation (Bremermann and Pickering, 1983, Bremermann and Thieme, 1989). Additionally, evolutionary cycling of parasite virulence and predator density can arise (Kisdi et al., 2013). Studies focussing on the evolution of host defence against a single enemy population, parasites (Hoyle et al., 2012 and Chapter 2), and multiple enemy populations, parasites along with predators (Chapter 3), have found that the presence of a predator population can lead to more diversity in the host population. In all of these evolutionary studies the predator was assumed to be immune, with the parasite only infecting the prey, i.e. the predator could consume an infected prey without becoming infected. One theoretical population dynamics study by Fenton and Rands (2006) has considered a population where the predators are no longer immune to infection, finding that manipulation of the prey, acting as the intermediate host, by the parasite can lead to oscillations in population densities. Despite this work, there is yet to be a formal theoretical analysis on the evolutionary dynamics of host manipulation by parasites when the predator population is no longer immune to infection, therefore the drivers of manipulation are unknown.

In this chapter, I look at when the parasite population will invest in intermediate host manipulation when the predator, the parasite's final host, is no longer immune to infection. Here, the parasite can manipulate its prey to make them more conspicuous to the predator, increasing the chances of the parasite successfully reaching its final host. Considering the evolution of the parasite population, I determine when it is beneficial for the parasite to invest in higher levels of intermediate host manipulation, as this comes at a cost to the parasites' spore production.

5.2 Model

An intermediate host-parasite model with a predator population (final host) was used, similar to the model studied by Fenton and Rands (2006). They developed population dynamic models (with Holling Type I, II and III responses) to investigate the impact of varying the level of manipulation on the predator-prey population dynamics. I extend upon this previous work by including evolutionary dynamics (using adaptive dynamics) and a trade-off between manipulation and spore production in the parasite population to determine when manipulation is likely to evolve if it is a costly trait for the parasite population to invest in. Additionally, I look at the impact of varying other ecological parameters in the system to determine how this impacts the evolutionary outcome of the system. The model is given by the following ordinary differential equations:

$$\frac{dS}{dt} = aS(1 - qS) - \rho_S(S, I)(P_S + P_I) - \frac{\beta\lambda S P_I}{\mu} - bS \tag{5.1}$$

$$\frac{dI}{dt} = \frac{\beta\lambda S P_I}{\mu} - (b + \alpha)I - \rho_I(S, I)(P_S + P_I) \tag{5.2}$$

$$\frac{dP_S}{dt} = \theta\rho_S(S, I)P_S - \rho_I(S, I)P_S - dP_S \tag{5.3}$$

$$\frac{dP_I}{dt} = \rho_I(S, I)P_S - P_I(d + \alpha_P) \tag{5.4}$$

where S, I, P_S and P_I are the densities of susceptible prey, infected prey, susceptible predators and infected predators, respectively. The parameters are defined in Table 5.1. In this model, the prey act as intermediate hosts for the parasites and the predators act as final hosts for the parasites. When an intermediate or final host is infected, it moves from the susceptible to the respective infected class (at rate β for the prey) with the predator becoming infected after consumption of an infected prey. I assume that there is no recovery (susceptible-infected [SI] model) and no reproduction for the intermediate and final hosts once they have been infected (see Figure 5.1 for a schematic

diagram of this model). Hence the infected prey are only removed through natural death, parasite-induced death or predation ($b+\alpha+c\phi(P_S+P_I)$) and the infected predators are only removed through natural death or parasite-induced death ($d+\alpha_P$). The parasites produce spores in their final host which are then released into the environment where they die or are consumed by prey (equivalent to $\frac{\lambda P_I}{\mu}$). We can assume the dynamics of this external parasite stage are relatively fast so it is not modelled explicitly (Fenton and Rands, 2006). This varies from the transmission process in Chapters 2 and 3 where the infection was transmitted through contact with an infected individual, as here infection of prey is through consumption of spores. I do not explicitly model the P_I population directly consuming the S or I populations due to the infertility assumption once infected meaning that the P_I population grows through the direct transfer of the infected predators from the P_S population. I use a Holling Type I (linear) response for the predators, assuming that there is no limitation on predation. For the Type I model, $\rho_S(S,I) = cS$ and $\rho_I(S,I) = c\phi I$. Here, ϕ is the increase or decrease in predation rate suffered by infected individuals (manipulation) relating to the level of selective predation occurring ($\phi > 1$, the predators are selectively preying upon infected prey; $\phi < 1$, the predators are selectively preying upon susceptible prey; $\phi = 1$, the predators are preying upon both equally).

Within this main text, I present the baseline model where there is no recovery or infected reproduction and a Holling Type I predation term but the full model with infected reproduction and Type II predation is discussed in Boxes 5.3 and 5.4, respectively. Additionally, I focus on the parameter region where all four populations (S, I, P_S and P_I) coexist and have stable population dynamics for the Holling Type I response (this parameter region is shown in Figure 5.13; refer to Box 5.1 for more details on other coexistence regions). The parameter region selected ensures that the dynamics remain stable throughout my results for the Holling Type I response.

Focussing on selection for the parasites to evolve manipulation of the

Table 5.1: Parameter definitions and values used

Parameter	Definition	Default Value
a	Prey birth rate	2
K	Prey carrying capacity, $1/q$	2
q	Strength of intraspecific density dependence acting on prey	0.5
c	Baseline predation rate	1.2
θ	Conversion of predation into births of new predators	0.5
d	Predator death rate	0.5
b	Prey death rate	0.2
α	Virulence for prey (intermediate host)	1.3
α_P	Virulence for predator (final host)	1
ϕ	Increase/decrease in predation rate suffered by infected individuals (due to parasite manipulation)	1.1
λ	Rate at which parasites in predators produce infective stages, which are passed on to environment then die/consumed by prey (spore production)	0.6
μ	Parasite death rate	0.1
β	Parasite consumption rate by prey (transmission rate of parasites to prey)	0.8
ρ_S, ρ_I	Holling Type I, II or III response	Varies

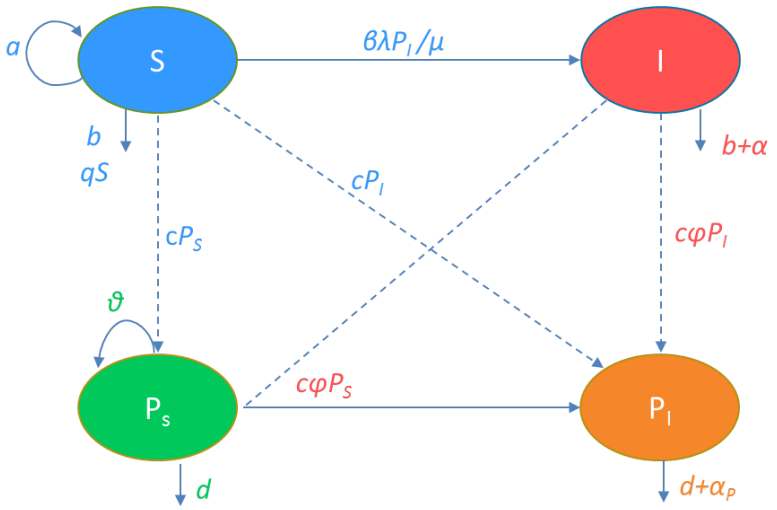


Figure 5.1: Flowchart diagram of model, where S, I, P_S and P_I are the densities of susceptible prey, infected prey, susceptible predators and infected predators, respectively (parameters are defined in Table 5.1). The dashed lines represent predation processes.

Box 5.1: Population dynamics

Throughout my results for Chapter 5, I focus on the region where all of the populations (S, I, P_S and P_I) coexist. There are regions where the intermediate host (S) exists alone, the intermediate host and predator (S, P_S) coexist or all four populations (S, I, P_S, P_I) coexist with cyclical dynamics.

Additionally, I focus on using a Type I Holling response where the fitness can be calculated using the method shown in Box 5.2. The parameter values selected ensure that the dynamics remain stable with real eigenvalues.

For the Type II and Type III Holling responses, imaginary eigenvalues can arise and it becomes intractable to obtain the explicit equilibrium population densities so the method in Box 5.2 can no longer be used. Hence, numerical simulations are used, following the method in Box 2.6 to determine the evolutionary outcome. In this case, limit cycles arise leading to cyclic dynamics in the population densities.

intermediate host through an increase in predation of infected intermediate hosts, I include a trade-off, $\lambda(\phi)$, between parasite spore production (which can also be thought of in terms of parasite growth rate) and manipulation of the intermediate host by the parasite. Here, the evolution of manipulation by the parasite is the main trait of interest. By increasing manipulation of the intermediate host, the parasites make them more prone to predation, thereby increasing the rate of transmission to the final host population, however, this comes at a cost to the parasites spore production levels as they are using up energy for manipulation. In this case, when the parasite increases manipulation of the intermediate prey, they constrain their levels of spore production and vice versa. The trade-off curve, $\lambda(\phi)$, is given by:

$$\lambda(\phi) = \lambda(\phi^*) - \frac{\lambda'(\phi^*)^2}{\lambda''(\phi^*)}(1 - e^{\frac{\lambda''(\phi^*)(\phi - 1.1)}{\lambda'(\phi^*)}}) \tag{5.5}$$

Here the primes denote derivatives, for example, $\lambda'(\phi^*) = \frac{\partial \lambda}{\partial \phi}\big|_{\phi=\phi^*}$ and (ϕ^*, λ^*) is the singular point. The trade-off curve is shown in Figure 5.2. The mutant

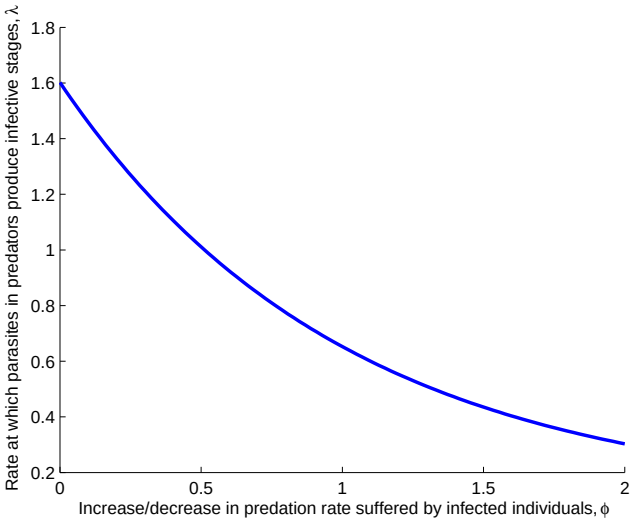


Figure 5.2: Trade-off curve between parasite spore production (λ) and manipulation (ϕ) where $(\phi^*, \lambda^*) = (1.1, 0.6), \lambda'(\phi) = -0.5$ and $\lambda''(\phi) = 0.5$.

strains vary in their trade-off strategies, such that higher host manipulation corresponds to lower spore production. For this model, the fitness equation for a mutant parasite invading a resident at equilibrium is given by the following (see Box 5.2 for computation of the fitness equation):

$$r(\hat{\lambda}, \hat{\phi}; \lambda, \phi) = -(d + \alpha_P)(b + \alpha + c\hat{\phi}(P_S + P_I)) + \frac{c\hat{\phi}\beta\hat{\lambda}SP_S}{\mu} \tag{5.6}$$

Here $\hat{\lambda}$ and $\hat{\phi}$ denote the mutant traits. When $r > 0$, the mutant population has positive fitness and can invade the resident population, when $r < 0$, the mutant population has negative fitness and cannot invade the resident population and when $r = 0$, the mutant population has zero fitness. The fitness equation, r, can be defined in terms of the basic reproductive ratio (R_0) as it is sign equivalent to $R_0 - 1$. The first term in equation 5.6 is the rate of removal of the infected final host population (through background mortality and parasite-induced death rate; $d + \alpha_P$) and the second term is

the rate of removal of the infected intermediate host population (through background mortality, parasite induced death rate and predation; $b + \alpha + c\phi(P_S + P_I)$). The additional third term is the net infection rate of predators (through consumption of infected prey; $c\phi P_S$) multiplied by the net infection rate of susceptible intermediate hosts (through intake of parasite; $\frac{\beta\lambda S}{\mu}$). Note that the R_0 value is given by:

$$R_0 = \frac{c\phi P_S}{b + c\phi(P_S + P_I)} \frac{\lambda}{d} \frac{\beta P_I}{\mu + \beta(S + I)}$$

where, the first term is the transmission of parasites from infected intermediate hosts to the predators, the second term is the net rate of parasite spore production and the third term is the infection process of the prey population (Fenton and Rands, 2006). The following results focus on the region where all four populations coexist.

5.3 Results

5.3.1 Population densities

I begin by looking at the evolutionary outcome of the system by looking for evolutionary singular points which occur when the gradient of the fitness equation is zero:

$$\frac{\partial r}{\partial \hat{\phi}} \mid_{\hat{\phi}=\phi} = -c(d + \alpha_P)(P_S + P_I) + \frac{c\beta(\lambda + \phi\lambda')SP_S}{\mu} = 0 \tag{5.13}$$

The points which the population evolves towards and stays at are known as continuously stable strategy (CSS) points. By re-arranging for the manipulation term (ϕ) and fixing the trade-off slope as $\lambda'(\phi^*) = -0.5$ (also fixed the singular point as (ϕ^*, λ^*)=(1.1, 0.6) and curvature as $\lambda''(\phi^*) = 0.5$ for my results in order to determine what will happen when there is a change in

Box 5.2: Fitness equation

To obtain the fitness equation for a mutant population trying to invade a resident equilibrium, I begin by considering a resident population which is at equilibrium and look at the possibility of invasion by a rare mutant. I use the stability of this resident-mutant equilibrium to calculate the fitness for the mutant population. The following are the equations for the mutant infected populations:

$$\frac{d\hat{I}}{dt} = \frac{\beta\hat{\lambda}S\hat{P}_I}{\mu} - (b+\alpha)\hat{I} - c\hat{\phi}\hat{I}(P_S + P_I) \tag{5.7}$$

$$\frac{d\hat{P}_I}{dt} = c\hat{\phi}\hat{I}P_S - (d+\alpha_P)\hat{P}_I \tag{5.8}$$

For the Holling Type I model, the fitness equation for a mutant parasite invading a resident equilibrium is obtained by taking the determinant of the negative Jacobian matrix, evaluated at the resident-only equilibrium, which gives:

$$-\begin{vmatrix} -(b+\alpha) - c\hat{\phi}(P_S+P_I) & \frac{\beta\hat{\lambda}S}{\mu} \\ c\hat{\phi}P_S & -(d+\alpha_P) \end{vmatrix}$$

$$r(\hat{\lambda},\hat{\phi};\lambda,\phi) = -(d+\alpha_P)(b+\alpha+c\hat{\phi}(P_S+P_I)) + \frac{c\hat{\phi}\beta\hat{\lambda}SP_S}{\mu} \tag{5.9}$$

Similarly, for the Holling Type II model, the equations for the mutant populations are now given by:

$$\frac{d\hat{I}}{dt} = \frac{\beta\hat{\lambda}S\hat{P}_I}{\mu} - (b+\alpha)\hat{I} - \frac{c\hat{\phi}\hat{I}(P_S+P_I)}{1+ch_SS+c\hat{\phi}h_I\hat{I}} \tag{5.10}$$

$$\frac{d\hat{P}_I}{dt} = \frac{c\hat{\phi}\hat{I}P_S}{1+ch_SS+c\hat{\phi}h_I\hat{I}} - (d+\alpha_P)\hat{P}_I \tag{5.11}$$

The negative determinant of the Jacobian matrix, evaluated at the resident-only equilibrium, for the Type II model is:

$$-\begin{vmatrix} -(b+\alpha) - \frac{c\hat{\phi}(P_S+P_I)}{1+ch_SS+c\hat{\phi}h_I\hat{I}} + \frac{(P_S+P_I)c^2\hat{\phi}^2h_I\hat{I}}{(1+Sch_S+c\hat{\phi}h_I\hat{I})^2} & \frac{\beta\hat{\lambda}S}{\mu} \\ \frac{c\hat{\phi}P_S}{1+ch_SS+c\hat{\phi}h_I\hat{I}} - \frac{c^2\hat{\phi}^2h_I\hat{I}P_S}{(1+ch_SS+c\hat{\phi}h_I\hat{I})^2} & -(d+\alpha_P) \end{vmatrix}$$

$$\begin{aligned} r(\hat{\lambda},\hat{\phi};\lambda,\phi) = -(d+\alpha_P)\Bigg(b+\alpha+\frac{c\hat{\phi}(P_S+P_I)}{1+ch_SS+c\hat{\phi}h_I\hat{I}} - \frac{c^2\hat{\phi}^2h_I\hat{I}(P_S+P_I)}{(1+ch_SS+c\hat{\phi}h_I\hat{I})^2}\Bigg) \\ +\frac{\beta\hat{\lambda}S}{\mu}\Bigg(\frac{c\hat{\phi}P_S}{1+ch_SS+c\hat{\phi}h_I\hat{I}} - \frac{c^2\hat{\phi}^2h_I\hat{I}P_S}{(1+ch_SS+c\hat{\phi}h_I\hat{I})^2}\Bigg) \end{aligned} \tag{5.12}$$

These equations are sign equivalent to the fitness equation computed using the dominant eigenvalue of these respective Jacobian matrices (see Box 1.1 in Chapter 1 for proof). Hence I use these as a fitness proxy to determine the fitness in my analysis.

the population densities), this equation becomes:

$$\phi = 2\lambda - \frac{2\mu(d+\alpha_P)(P_S+P_I)}{\beta S P_S} \tag{5.14}$$

showing that for a population at a CSS point, if the final host population $(P_S + P_I)$ increases, manipulation will decrease and if the susceptible intermediate host population (S) increases, manipulation will increase (see Figure 5.3). This can also be seen if we initially assume that the parasites have no manipulation ($\phi = 0$) and consider when the parasites will evolve manipulation ($\frac{\partial r}{\partial \hat{\phi}} \mid_{\hat{\phi}=\phi=0} > 0$; positive selection gradient at $\phi = 0$). Looking at equation 5.13, we can see that manipulation is likely to evolve when there is an increase in the susceptible intermediate host population and a decrease in the final host population. Hence, the parasite's key determinant to evolving manipulation depends on the predator population density, if it increases, they will lower their manipulation and vice versa. The infected intermediate host population (I) does not play a role in the equation showing that their population density does not directly impact the evolution of parasite manipulation as discussed previously. Thus, from the equations, we can see that the parasites will invest more in manipulation when there is a growth in the intermediate host population with a decrease in the final host population and less when there is a decrease in the intermediate host population with a rise in the final host population.

5.3.2 Varying the trade-off curve

The trade-off curve, specifically whether evolving manipulation is increasingly or decreasingly costly, affects the evolution of parasite manipulation. When $\lambda''(\phi) > 0$, the trade-off curve is a convex curve with decreasing costs and when $\lambda''(\phi) < 0$, the trade-off curve is a concave curve with increasing costs (see Figure 5.4). Given increasing costs (low trade-off curvature), the parasites may evolve significant manipulation but given decreasing costs

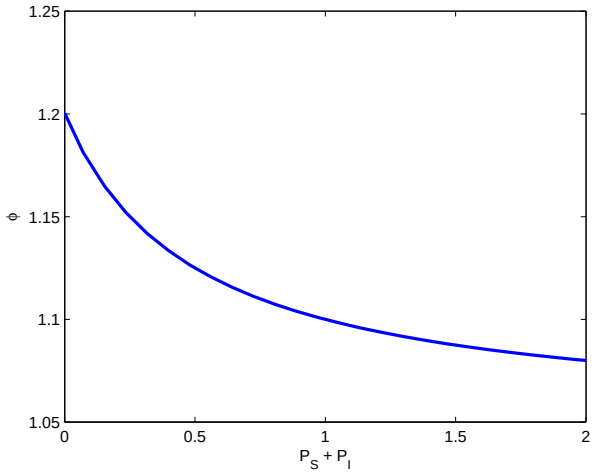


Figure 5.3: Graphical representation of equation 5.14 showing that manipulation (ϕ) decreases as the predator population ($P_S + P_I$) increases, using the parameter values given in Table 5.1.

(high trade-off curvature), the parasites may evolve lower manipulation levels (see Figure 5.4). For very strong decelerating curvature values, the parasites may continue to invest in a stable but small amount of manipulation. Additionally, for $\lambda''(\phi) > 0.9091$, repellors start to arise at high levels of manipulation (as shown in Figure 5.5) so the population will evolve away from high levels of manipulation depending upon initial conditions. Here, the evolutionary stability (ES) and convergence stability (CS) conditions no longer hold (see Section 5.5 for more on these conditions). The value of $\lambda''(\phi)$ does not have an explicit effect on the MI condition (given by equation 5.16). In the following results, $\lambda''(\phi) = 0.5$ is used as the trade-off curvature allowing the parasites to be able to evolve either low or high manipulation (as this is an intermediate manipulation level as can be seen in Figure 5.4). See Bowers et al. (2005) and Kisdi (2006) for more on trade-offs.

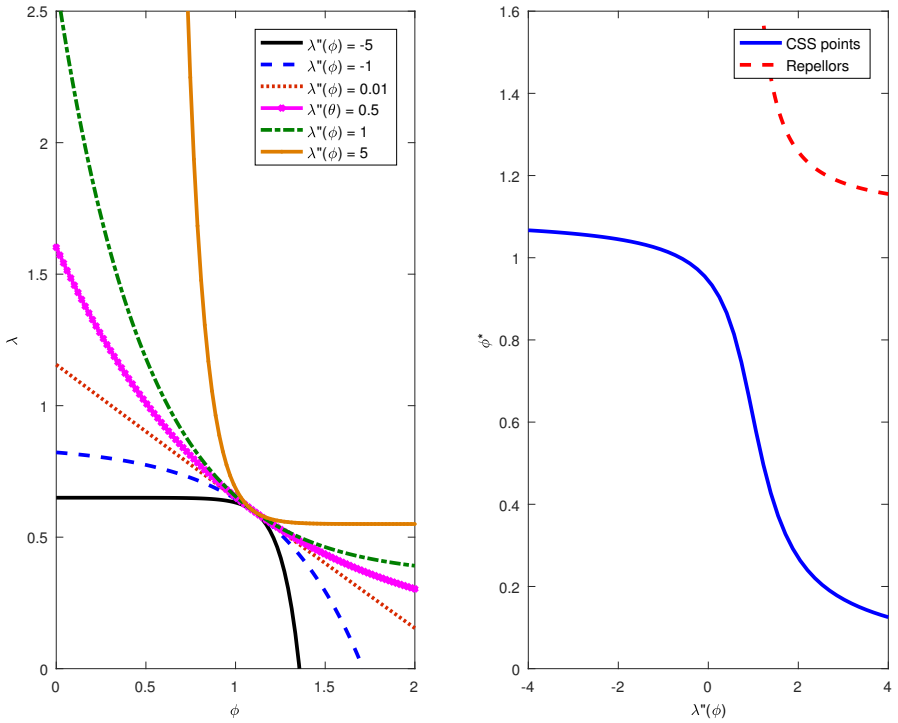


Figure 5.4: Trade-off curve, $\lambda(\phi)$, for varying trade-off curvature values ($\lambda''(\phi)$), where $(\phi^*, \lambda^*) = (1.1, 0.6)$. CSS manipulation rate (ϕ^*) and repellors for varying $\lambda''(\phi)$ values. Other parameters are given in Table 5.1.

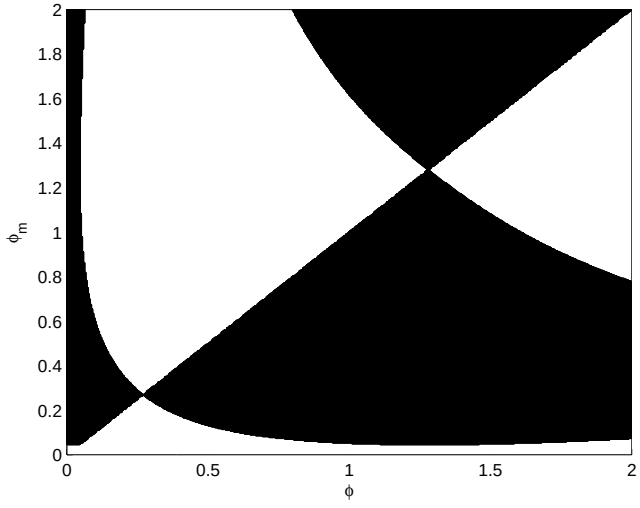


Figure 5.5: Pairwise invasibility plot (PIP) showing CSS and repellor manipulation rates (ϕ), where $\lambda''(\phi) = 2$. Other parameters are given in Table 5.1.

5.3.3 Virulence for the intermediate and final hosts

As virulence for the intermediate host (α) increases, the parasites lower their manipulation of the intermediate hosts, increasing their spore production. Here, as α increases, the population of final hosts increases as the intermediate host population decreases due to the higher parasite-induced death rate (shown in Figure 5.6a). As the infected intermediate hosts are more likely to die from infection than predation, the infected predator population declines as their susceptible population grows because they are preying predominantly upon susceptible intermediate hosts. A large final host population makes predation more likely without any additional manipulation so the parasites invest more in spore production, rather than manipulation (see Figure 5.7). Intuitively, since high virulence drives intermediate host numbers down, we may expect the parasites to increase manipulation in order to maintain transmission but as this also leads to a larger final host population, the risk of predation is already sufficiently high. Therefore, as α increases, the parasites invest less in manipulation. This varies from standard host-parasite models without predation in which an increase in virulence typically leads to a larger susceptible population, due to a reduced infectious period and exposure risk, therefore, we see a clear effect of predation relative to standard models (Boots and Haraguchi, 1999, Miller et al., 2007).

As virulence for the final host (α_P) increases, the predator population once again increases. We may expect it to decrease but by increasing the virulence for the predator, the infected predators are removed from the population, benefitting the non-infected predator population, thereby allowing the susceptible predator population to increase leading to a decline in the intermediate host population (shown in Figures 5.6b and 5.7). Since the predator population is large, the parasites lower their levels of manipulation. This can also be seen from equation 5.14 showing that as α_P increases, ϕ decreases. In summary, I find that manipulation is most likely in parasites that are less virulent to both the intermediate and final hosts. These results

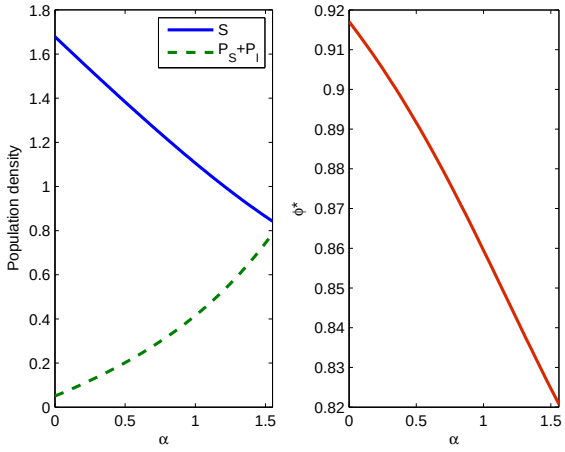
hold regardless of whether virulence is higher for the intermediate or final host population (refer to Figure 5.8).

The parameters for prey death rate (b), intraspecific density dependence (q), baseline predation rate (c), conversion of predation into births of new predators (θ) and parasite death rate (μ) behave similarly to the virulence terms as increases in these parameters lead to a decline in the intermediate host population and an increase in the final host population so the parasites invest less in manipulation and more in spore production (shown in Figure 5.9).

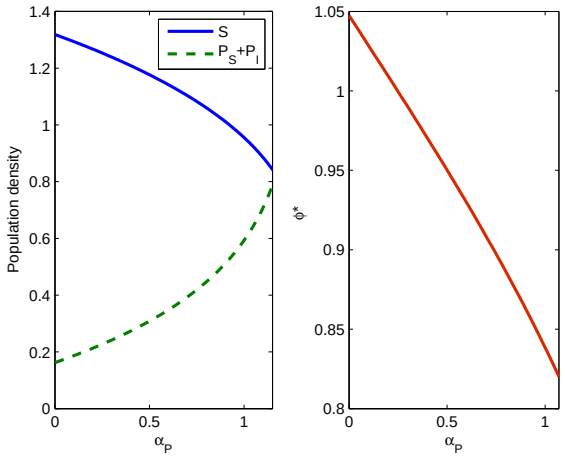
5.3.4 Parasite consumption rate by prey and predator death rate

As parasite consumption rate by prey (β) increases, the parasites lower their spore production, increasing their manipulation of the intermediate hosts. Here, as β increases, the intermediate host population increases and the final host population decreases because increasing the transmission rate of the parasites to the prey leaves less intermediate hosts for the predators to prey upon so their population declines (shown in Figure 5.10a). Since there are fewer predators, the risk of predation is low making manipulation more beneficial. Using equation 5.14, we can also see that as β increases, manipulation (ϕ) increases.

Similarly, as predator death rate (d) increases, the parasites increase their manipulation of the intermediate host (shown in Figure 5.10b). Looking at equation 5.14, we may expect ϕ to decrease as d increases but in this case the population densities are having a larger effect on the parasites investment strategy. Clearly, as d increases, the final host population declines leading the parasites to invest more in manipulation to ensure transmission to the final host.

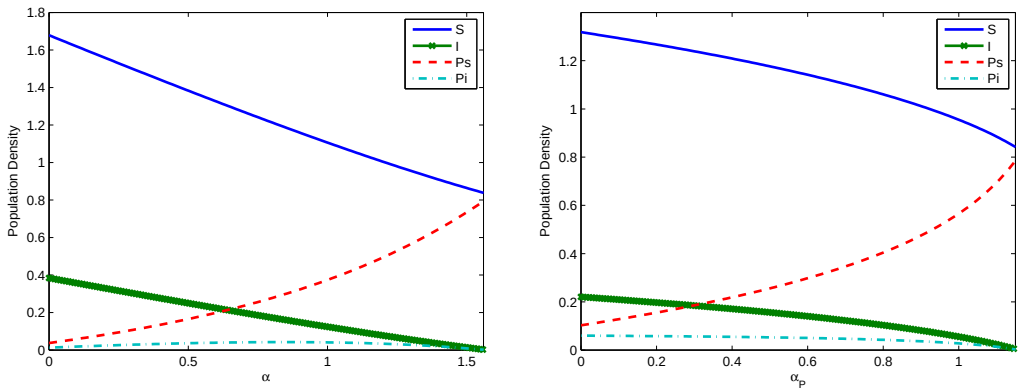


(a) Varying α.



(b) Varying α_P.

Figure 5.6: Population densities and ϕ^*, using parameters given in Table 5.1.



(a) Varying α. (b) Varying α_P.

Figure 5.7: Population densities, using parameters given in Table 5.1.

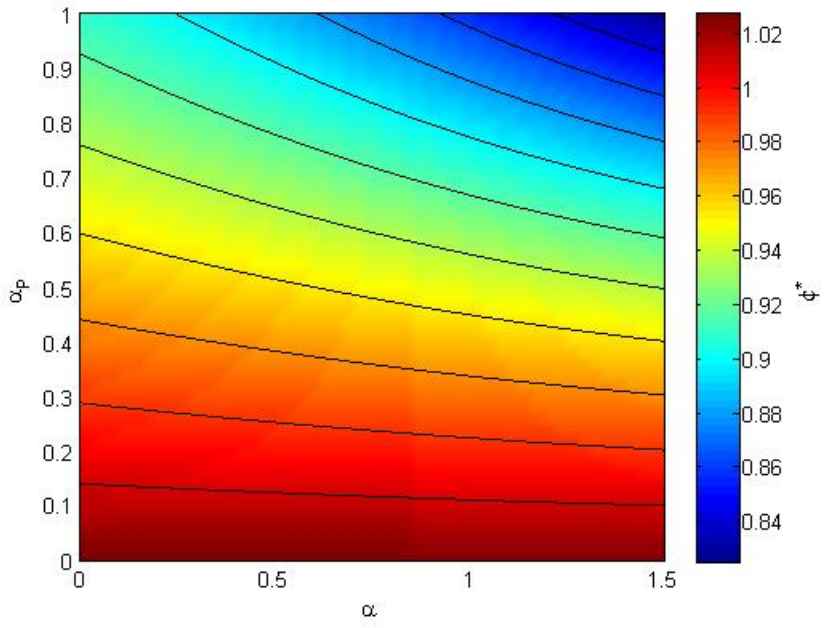
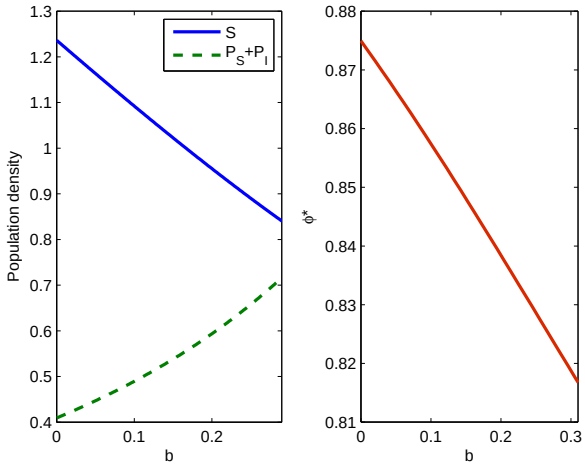
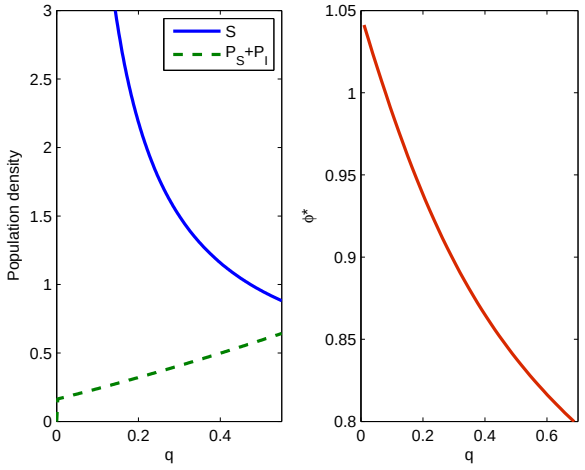


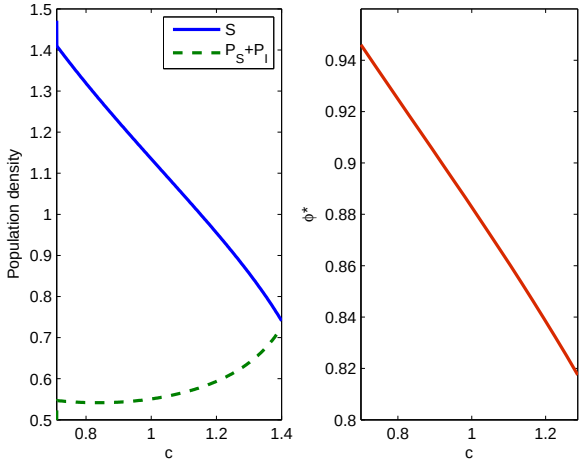
Figure 5.8: 2D-plot for α and α_P, using the parameter values given in Table 5.1 showing as α or α_P increase, the parasites invest less in manipulation, regardless of whether $\alpha > \alpha_P$, $\alpha_P > \alpha$ or $\alpha_P = \alpha$.



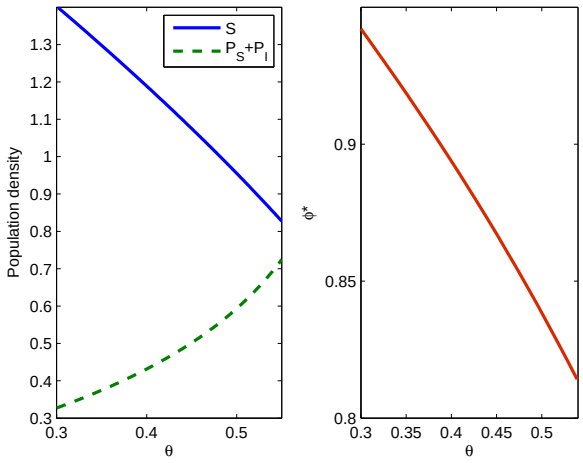
(a) Varying b.



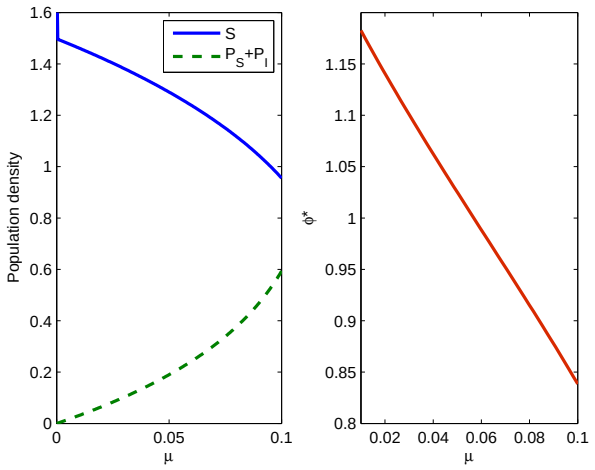
(b) Varying q.



(c) Varying c.



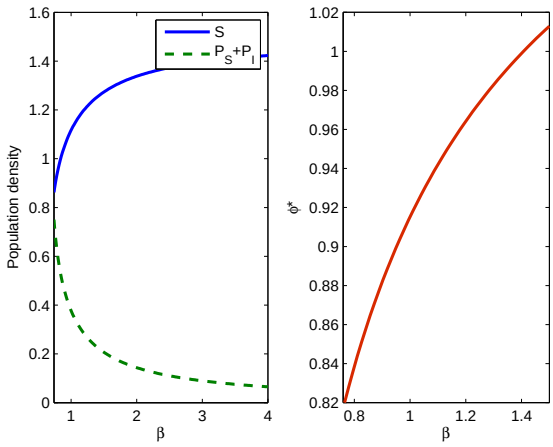
(d) Varying θ.



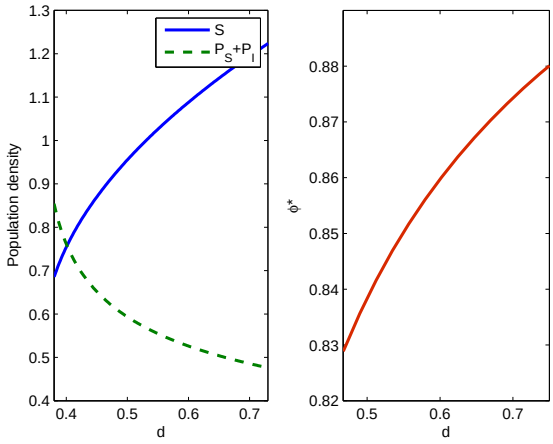
(e) Varying μ.

Figure 5.9: Population densities and CSS manipulation rate (ϕ^*), using the parameter values given in Table 5.1.

The parameter for prey birth rate (a) behaves similarly to β and d as increasing this parameter leads to lower spore production and greater manipulation by the parasite as the intermediate host population grows and the final host population declines (shown in Figure 5.12).

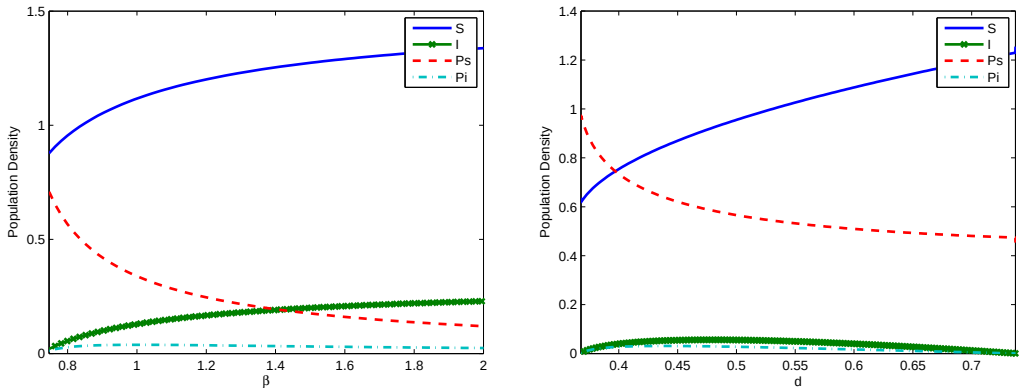


(a) Varying β.



(b) Varying d.

Figure 5.10: Population densities and ϕ^*, using parameters given in Table 5.1.



(a) Varying β.

(b) Varying d.

Figure 5.11: Population densities, using parameters given in Table 5.1.

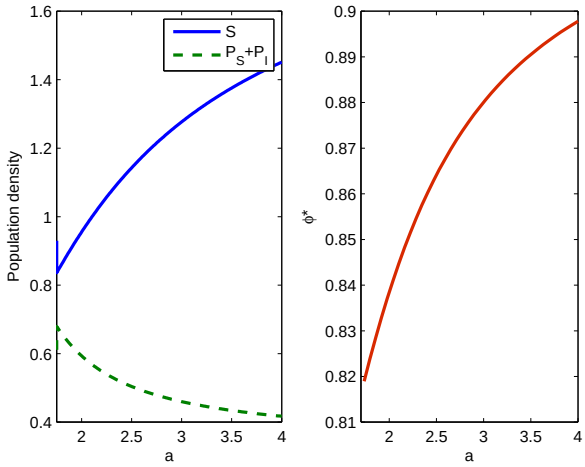


Figure 5.12: Population densities and CSS manipulation rate (ϕ^*) for varying a, using the parameter values given in Table 5.1.

5.4 Summary of results

I have focussed on determining when the parasites will evolve greater manipulation correspondingly constraining their spore production and when they will evolve less manipulation correspondingly increasing their spore production. Generally, the parasites evolution strategy is driven by the risk of predation faced by the intermediate host population as this relates to how beneficial it is for the parasites to invest in manipulation. I used the population densities of the intermediate and final hosts to determine whether there is a low or high risk of predation. The parasites increase manipulation of the intermediate host when there is an increase in the intermediate host population (S) and a decline in the final host population ($P_S + P_I$). Since there is only a small predator population, the risk of predation is low so to ensure that infected hosts are consumed, the parasites increase manipulation making the intermediate hosts more conspicuous to the predators. However, the parasites invest less in manipulation if there is an increase in the final host population ($P_S + P_I$) and a decrease in the intermediate host popula-

tion (S) due to infection or predation. Since in this case there are many predators, the intermediate hosts are already facing a high risk of predation so the parasites do not need to invest in manipulation and can instead focus on their spore production.

Interestingly, the infected intermediate host population (I) does not directly impact the parasites evolution strategy, so regardless of whether this population is small or large, the parasite will invest in manipulation according to the density of the other populations. The infected intermediate population does of course have an effect on the other population densities thereby having an indirect effect on the evolution of manipulation. The other population densities have a clear impact on the parasites' evolution strategy as they are directly linked to the parasites life-cycle since the parasites produce spores in the final hosts and rely on consumption of spores by the susceptible intermediate hosts. The predator population density is vital in determining whether manipulation will evolve as it directly impacts the risk of predation so it clearly has an effect on the parasites evolution strategy. Hence, the parasites invest more in manipulation, lowering their spore production, if there is a small risk of predation (increase in S; decrease in $P_S + P_I$). The parasites invest less in manipulation, increasing their spore production, if there is a high risk of predation (decrease in S; increase in $P_S + P_I$).

5.5 Branching

Next, I consider the possibility of diversity occurring through evolutionary branching in the model. The occurrence of such behaviour would lead to two coexisting parasite populations with different traits such as one parasite population with high levels of manipulation and low spore production and another with high levels of spore production and low levels of manipulation. I first compute the evolutionary stability (ES, meaning the population will stay at this point once reached as no nearby mutants can invade) and convergence

stability (CS, meaning the population will converge to this point when close to it) conditions to determine if branching is possible. Branching occurs when there is CS but no ES. The ES condition is given by:

$$\frac{\partial^2 r}{\partial \hat{\phi}^2} \mid_{\hat{\phi}=\phi} = \frac{2c\beta\lambda' SP_S + c\phi\beta\lambda'' SP_S}{\mu} < 0 \tag{5.15}$$

The MI (mutual invasibility) condition is given by:

$$\begin{aligned} \frac{\partial^2 r}{\partial \hat{\phi} \partial \phi} \mid_{\hat{\phi}=\phi} &= -c(d+\alpha_P)(P'_S + P'_I) \\ + \frac{c\beta\lambda SP'_S + c\beta\lambda S'P_S + c\phi\beta\lambda' SP'_S + c\phi\beta\lambda' S'P_S}{\mu} &< 0 \end{aligned} \tag{5.16}$$

The CS condition is then given by ES+MI (equations $[5.15 + 5.16] < 0$).

Branching does not occur in this model for the parameter values given in Table 5.1, as shown in Figures 5.13 and 5.14. Below each line the relevant condition is met, i.e. attractors occur below the ES and CS lines, repellors occur above both lines and garden of eden points occur between the two lines. A thorough exploration of parameter space was carried out to determine whether any branching occurs in this system. The numerical analysis showed that no branching occurred, hence, no diversity arises in the parasite population so it will tend to evolve towards either high levels of manipulation with low spore production or low manipulation with high spore production.

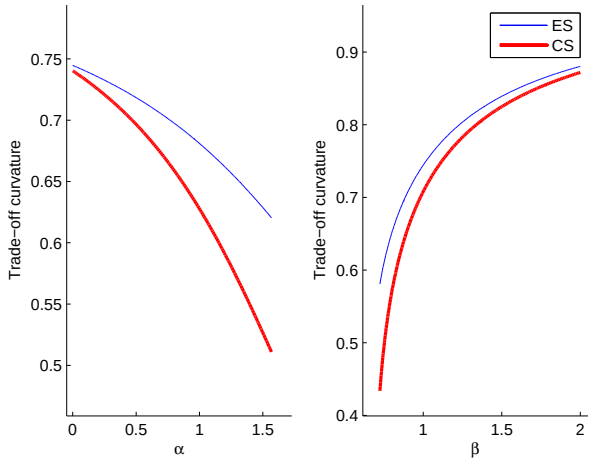
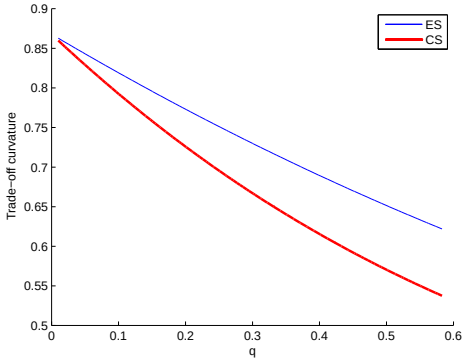
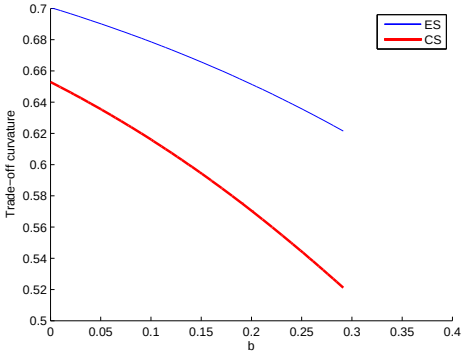


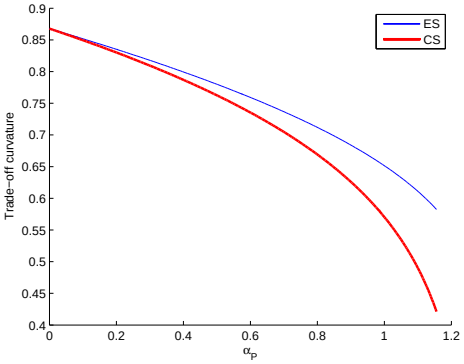
Figure 5.13: Evolutionary outcome regions for varying α and β, where $(\phi^*, \lambda^*) = (1.1, 0.6)$. Other parameters are given in Table 5.1. Below each line the relevant ES or CS condition is met.



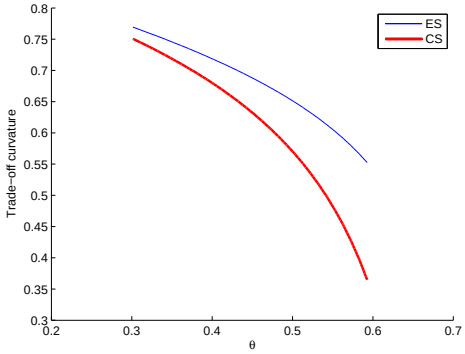
(a) Varying q.



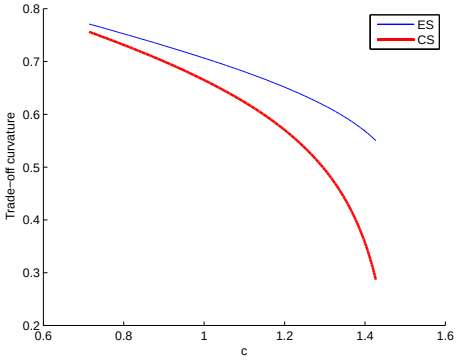
(b) Varying b.



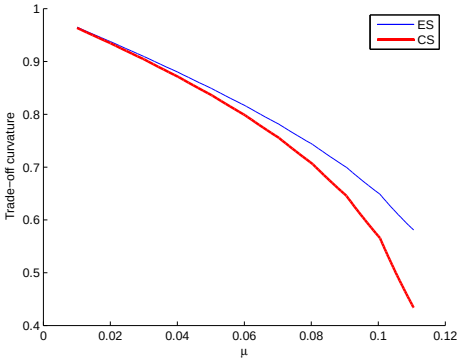
(c) Varying α_P.



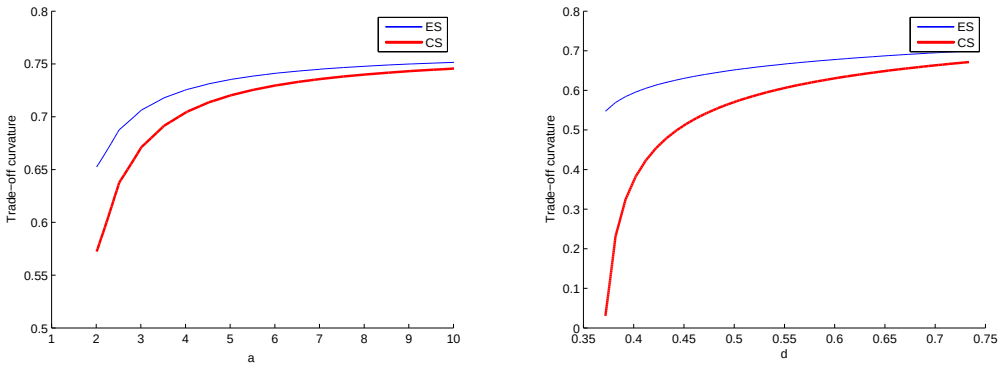
(d) Varying θ.



(e) Varying c.



(f) Varying μ.



(g) a. (h) d.

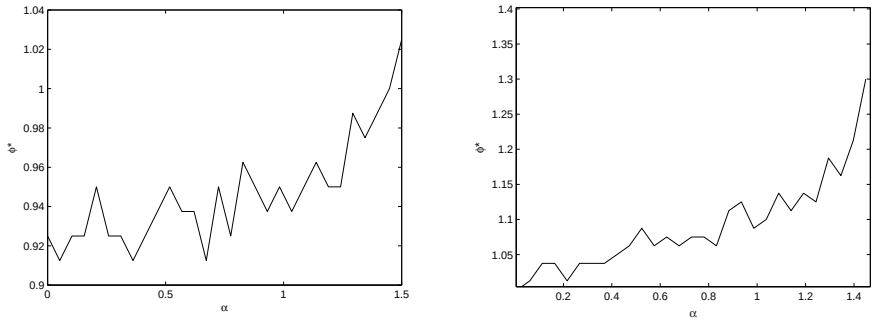
Figure 5.14: Evolutionary outcome regions, where $(\phi^*, \lambda^*) = (1.1, 0.6)$. Other parameters are given in Table 5.1. Below each line the relevant ES or CS condition is met.

Box 5.3: Holling Type II model

I consider altering the predators functional response to a Holling Type II (hyperbolic) response, giving the predator a decelerating intake rate as the number of prey increases because it is limited by its capacity to intake prey and by the time it takes to consume them. For the Type II model, $\rho_S(S,I) = \frac{cS}{1+ch_SS+c\phi h_II}$ and $\rho_I(S,I) = \frac{c\phi I}{1+ch_SS+c\phi h_II}$, where h_S and h_I are the handling times for susceptible and infected prey, respectively (also used by Fenton and Rands (2006)). The fitness equation now becomes (see Box 5.2 for computation of the fitness equation):

$$r = -(d+\alpha_P)\left(b+\alpha+\frac{c\phi(P_S+P_I)}{1+ch_SS+c\phi h_II} - \frac{c^2\phi^2h_II(P_S+P_I)}{(1+ch_SS+c\phi h_II)^2}\right) + \frac{\beta\lambda S}{\mu}\left(\frac{c\phi P_S}{1+ch_SS+c\phi h_II} - \frac{c^2\phi^2h_IIP_S}{(1+ch_SS+c\phi h_II)^2}\right) \tag{5.17}$$

For the Type II case, it becomes intractable to obtain explicit population densities as population cycles arise (with imaginary eigenvalues when solving for the fitness equation; see Box 5.1) so we rely on the results produced by running numerical simulations (see Figure 5.15 and Box 2.6). Here, the results are similar to the Type I results as the parasites evolve greater manipulation if there is a decline in the final host population with an increase in the intermediate host population and vice versa. The parasites increase their manipulation to higher levels compared to the Type I model as handling time increases in order to ensure that a sufficient number of infected prey are consumed by the predators (see Figure 5.15). This rise in manipulation as handling time increases to maintain parasite persistence has also been found by Fenton and Rands (2006). Overall, there is no significant difference by altering the predators functional response as the results remain similar to the Type I model.



(a) Type I.

(b) Type II.

Figure 5.15: Holling simulations for α, where $h_S = 0.35, h_I = 1.5$. Other parameters given in Table 5.1.

Box 5.4: Infected fecundity

To determine if the addition of infected fecundity will impact my results, I introduce an infected fecundity term (f), to the $\frac{dS}{dt}$ equation in the model, as follows:

$$\frac{dS}{dt} = aS(1-qS) + fI - cS(P_S + P_I) - \frac{\beta\lambda SP_I}{\mu} - bS \tag{5.18}$$

Here, as f approaches zero, the proportion of infected intermediate hosts able to reproduce decreases and when $f = 0$, the infected population is completely sterile. As f increases, the number of intermediate hosts increases so the parasites invest more in manipulation to ensure that enough infected intermediate hosts are preyed upon (see Figure 5.16). This changes for high virulence for intermediate hosts (α) and for low parasite consumption rate by prey (β) values as here when f increases, the parasites invest less in manipulation due to the growing predator population. Apart from these slight changes in the amount of investment in manipulation, the results generally remain the same as the model with no infected fecundity. So by using the assumption that all infected hosts are sterile ($f = 0$), the model results are not altered. Here, for simplicity, I assumed that the infected intermediate hosts do not have an effect on the density dependence term as when f increases, the parasites invest more in manipulation and the predator population is larger so they are likely to be removed quickly from the population.

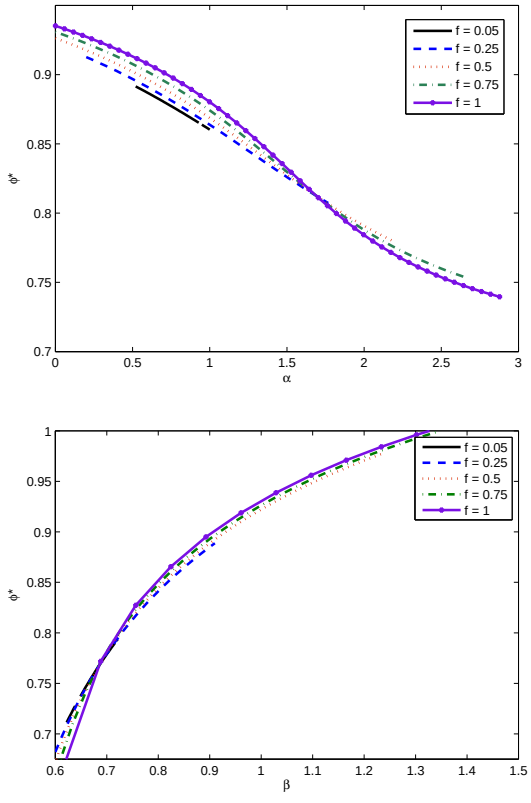


Figure 5.16: Varying levels of f for α and β, using parameters in Table 5.1.

5.6 Discussion

I have found that parasites evolve manipulation according to the risk of predation in their environment. Since parasites increase manipulation in order to increase their chances of transmission to the final host, the size of the final host population is vital in determining whether it is beneficial for the parasites to invest in manipulation. Given a decline in the predator population, it is beneficial for the parasites to invest in manipulation due to the lowered risk of predation faced by the intermediate hosts. To ensure that the parasites reach their final host, the parasites increase manipulation making the infected hosts more conspicuous to predators and thus more likely to be consumed, thereby increasing the net transmission rate of the disease. The presence of a large predator population makes it less likely for the parasites to invest in manipulation as there is already a high chance of predation to occur without any additional manipulation. Hence, my results have shown that the parasite population will evolve greater levels of manipulation when there is a decrease in the final host population and lower levels of manipulation when there is an increase in the final host population.

The results showed a decline in the intermediate host population and a rise in the final host population when the longevity of the infected intermediate hosts decreased (through increasing virulence (α) or mortality (b)) or the passive transmission rate increased (through increasing baseline predation rate (c) or conversion of predation into births of new predators (θ)) leading the parasites to invest less in manipulation. Intuitively, we may expect the parasites to increase manipulation as the longevity of infected intermediate hosts decreases to ensure that they reach their final host before their intermediate host dies (Poulin, 1994). However, in our mathematical model we have shown that the parasites actually invested less in manipulation, showing that the size of the final host population plays a larger role than the infected intermediate population (which we have shown has no direct impact on the evolution of host manipulation) on the parasites evolution strategy.

Greater manipulation has been shown to allow for the persistence of highly virulent parasites, suggesting that as virulence increases, the parasites should increase manipulation (Fenton and Rands, 2006). Taking into consideration the ecological feedbacks from the predator population on the evolution of manipulation and the costs of evolving manipulation, the growth of the final host population meant that the parasites could focus less on manipulation and more on spore production in these two cases.

The parasites invested less in manipulation when there were high predator birth rates as this led to an increased risk of predation for the susceptible intermediate hosts. Similarly, but rather non-intuitively, an increase in virulence for the predators (α_P), lead to a rise in the predator population as the infected predators were removed from the population, benefitting the susceptible predators, leading to a higher risk of predation so the parasites once again invested little in manipulation. In contrast, increasing the parasite consumption rate by prey (β) or the predator death rate (d) both lead to a decline in the predator population causing the parasites to invest more in manipulation to ensure transmission to the final host. Overall, the results show that given an increase in the prey population and a decrease in the predator population, the parasites invest more in manipulation due to the low risk of predation and vice versa.

Previous work on parasite evolution has shown that parasite diversity can increase (Morozov and Adamson, 2011, Morozov and Best, 2012) and evolutionary cycling can arise (Kisdi et al., 2013) in the presence of a predator population. Standard models of parasite evolution find that a single parasite strain should always be optimal (Bremermann and Pickering, 1983 but see Best and Hoyle, 2013 for counter-examples). Whilst considering whether two parasite populations could coexist with different manipulation strategies in this model, i.e. one population investing highly in manipulation and one investing less in manipulation, I did not find any such diversity arising in the parasite population. Hence these results suggest it is unlikely to find

coexisting parasite populations with varying manipulation strategies. Due to the dependence of the evolution of manipulation on the predator population density, it is clear that the parasite population will evolve either low or high levels of manipulation accordingly. Using a Holling Type II response, constraining the intake of prey by predators, leads to the parasites investing more in manipulation to ensure enough infected prey are consumed, though the general results remained the same (see Box 5.3). The inclusion of infected fecundity for the prey population also did not alter my main results (see Box 5.4; for further analysis, we could similarly consider the inclusion of infected fecundity for the predator population). Overall, my results show the importance of the population densities, particularly the predator population, on the parasites investment in manipulation.

In nature, we do not see all parasite species evolving manipulative characteristics, suggesting that there is likely to be a cost to do so. Franceschi et al. (2010) found that parasites that develop rapidly do not induce behavioural changes in the host, whilst parasites that develop slowly manipulate the behaviour of their host, suggesting that investment in manipulation leaves less energy for parasite growth. Experiments carried out by Cressler et al. (2014) showed that parasites use energy going towards growth as a resource (Frost et al., 2008) and Vizoso and Ebert (2005) showed the existence of a parasite trade-off between spore production (horizontal fitness) and infected host offspring (vertical fitness). An example of costly manipulation is seen in the trematode, *Dicrocoelium dendriticum*, which uses ants as its second intermediate host manipulating them to climb to grass blade tops, making themselves highly susceptible to sheep and cattle, the parasites final hosts (Poulin, 1994). Usually all cercariae ingested by an ant are clones but one of them migrates to the ant's suboesophageal ganglion causing the ant to alter its behaviour (Wickler, 1976), whilst consequently becoming unable to infect the host and dying, clearly showing that the manipulation of host behaviour comes at a price. These studies highlight the costs that parasites face when investing

in manipulation. Furthermore, by removing the trade-off in our model, i.e. allowing the parasite population to evolve manipulation with no costs, it is likely that the parasites would evolve maximum levels of manipulation. This would confirm that there are most likely to be costs to evolving manipulation as we do not see all parasites in nature evolving highly manipulative traits. Using a trade-off where there are costs to evolving manipulation, my results show that the parasites are likely to evolve manipulation given low predator densities which can arise due to increases in parasite consumption rate by prey, predator death rate or prey birth rate.

The clear advantage of evolving manipulation arises when the predator is the final host for the parasite, making it beneficial for the parasite to manipulate their intermediate host to increase their chances of transmission to the final host through predation. For example, the protozoan parasite, *Toxoplasma gondii*, makes changes in its intermediate host, rats, limbic system (House et al., 2011, Mitra et al., 2013) making them more attracted to the scent of cat urine, increasing their chances of predation by their final host, cats (Weinersmith and Faulkes, 2014). Similarly, the acanthocephalan, *Pomphorynchus laevis*, parasitising the gammarid, *Gammarus pulex*, manipulates its hosts to have an escape response towards the water surface thus increasing the likelihood of predation by waterfowl, the final hosts of the parasite (Maure et al., 2013). It has been questioned whether such behavioural modifications seen in infected intermediate hosts are due to parasite manipulation or simply a by-product of infection. Addressing this, Levri (1999) showed that a parasite-induced change in the host behaviour of freshwater snails is due to parasitic manipulation and not a by-product of infection. Similarly, a field study on a fish parasite by Brown et al. (2001) showed that the presence and intensity of infection contributed to a modified behavioural response in the host. Hence, the behavioural changes seen in intermediate hosts seem to be evidently due to manipulation by the parasite. Additionally, in terms of predation, Lafferty (1992) suggests that there is often no selective pressure

on predators to avoid parasitised prey as the effects of parasites on definitive hosts are usually not large enough to counter the advantages associated with feeding on prey that are easy to locate and capture. This relates to my finding that given low virulence, the parasites invest more in manipulation as additional manipulation is needed to increase the selective pressure of the predators preying upon infected prey.

In terms of future work in this area, there are more complicated forms of parasite manipulation techniques that could be considered. Seppala and Jokela (2008) looked at two types of parasite manipulation, specific (aimed at a suitable predator) and nonspecific (not aimed at a particular predator) and found that in order to increase the likelihood of parasite transmission, nonspecific manipulation needs to increase the overall predation risk of infected hosts. Given a low initial predation risk, highly nonspecific manipulation strategies can be adaptive and given a high initial predation risk, manipulation needs to be more specific to increase parasite transmission success. Therefore, nonspecific host manipulation may evolve in nature, but the adaptive value of a certain manipulation strategy can vary among different parasite populations depending on the variation in initial predation risk. This agrees with my findings, as I found that the parasites alter their investment in manipulation depending on whether there is a decreasing or an increasing predator population as this relates to the risk of predation.

We could also consider a change in the parasites investment strategy depending on their age. For example, Parker et al. (2009) looked at when a parasite should manipulate its host, using a model where the parasites begin investing in predation enhancement once they are capable of establishing in their next host, so manipulation starts low then increases. A slightly different model to look at burden-dependent host manipulation (macroparasite model) to track the number of parasites a host is infected with could be studied (considered by Fenton and Rands, 2006), rather than a microparasite model (such as the one we have used in this chapter) which puts the host as either

susceptible or infected without explicitly tracking the number of parasites in the population. Another major aspect in this area which needs more insight is the parasite costs and mechanisms of manipulation as there is a poor understanding of this area (Poulin, 2010, Maure et al., 2013).

Although many studies have found evidence of parasite manipulation of intermediate host behaviour, future studies on parasite manipulation should also monitor the population densities, particularly the size of the predator population, as I have found that this relates to the risk of predation determining whether the parasite is likely to evolve manipulation. It would be interesting to see how these results compare to a study looking at the evolution of parasite manipulation in environments with varying risks of predation. In conclusion, predation has a large impact on the parasites' evolutionary strategy so the inclusion of a predator population is important when looking at the evolution of host-parasite systems.

Chapter 6

Discussion

6.1 Summary

I have studied the evolution of host-parasite systems whilst incorporating wider community complexity through the addition of a predator population. With the use of mathematical models, I have improved our understanding of how these populations interact and impact each other. I have determined the population dynamics which drive the evolution of host defence, as well as the evolution of host manipulation by parasites. Additionally, I have conducted experimental work to look at the evolution of host resistance and determine the existence for any evidence of trade-offs. By building upon this field and discovering new key insights into the underlying evolutionary dynamics in host-parasite systems, I have increased our understanding of such systems.

The inclusion of the predator population in the host-parasite models has shown that it has an impact on the evolution of host-parasite systems. Whilst focussing on the evolution of host resistance in the presence of a predator, I have found that the hosts vary their defence levels as the predation rate changes. The size of the predator population (relative to the size of the host population) relates to the risk of predation which plays a key role in determining when the hosts evolve resistance and when the parasites evolve

host manipulation. Hence, it is vital that such complexities are considered when studying such systems as it is likely that these interactions will occur in nature. In particular, I have highlighted the importance of population dynamics as the relative population densities have a large impact on the evolution of the host and parasite populations. As the evolution of host defence is determined by the risk and cost of infection relating to the infected host and predator populations, we have shown that the hosts maximise their levels of resistance at intermediate predation rates as this is where the combined risk and cost of infection is highest (Chapter 2).

Host defence against a particular enemy, parasites or predators, is driven by the composition of the surrounding enemy community, which is determined by the ratio of infected hosts to predators, and their relative growth rates. In general, the hosts build up defences against the enemy population posing a larger threat, with the growth rates coming into play when there is significant recovery and reproduction from infected hosts (Chapter 3). These results give us an insight into how the host species allocate resources between defences against parasite and predator populations. Additionally, this highlights the importance of recording population densities within experimental studies.

The risk of predation, determined by the predator population density, also played a key role in the evolution of host manipulation by parasites as it determined whether it is beneficial for the parasites to invest in manipulation. In particular, given a low risk of predation, parasites invest more in manipulation to maintain transmission to the final host and given a high risk of predation, parasites invest less in manipulation as they are likely to be preyed upon without any additional manipulation (Chapter 5). This work has uncovered new insights into the importance of population dynamics, whilst revealing the considerable impacts of adding predators to host-parasite models. Therefore, it is vital that wider ecological feedbacks are included in studies on evolution in systems, such as wildlife and managed populations,

where there are community dynamics at play.

Although the presence of the predator clearly increases the mortality faced by the hosts, it is different to simply increasing the mortality rate as the predator population is dynamic. This adds more complex ecological feedbacks to host-parasite systems and can give rise to unexpected evolutionary behaviours. For example, given higher recovery rates from infection, we may expect the hosts to lower their defences against parasitism but I found that the hosts increased their defences to parasites because the predator population declined faster than the infected population (Chapter 3). This shows that it is vital that we include a predator population when studying these systems.

In models without predation, defences against parasites are generally expected to be highest when there is a high exposure rate to disease with Boots and Haraguchi (1999) correspondingly showing that host resistance to highly virulent diseases is lower due to the reduced risk of exposure. I have found that this result holds for high predation rates but at low and intermediate predation rates, as virulence increased, I found that the hosts increased resistance due to the high cost and risk of being infected (Chapter 2). I also found this when defence against the infection constrained defence against predation, as here when virulence increased, the hosts increased their defences against the infection, rather than predation, due to the relatively smaller predator population (Chapter 3). This highlights the importance that the predator population has upon host-parasite evolution as it can reveal seemingly non-intuitive dynamics.

Whilst considering diversity in the host-parasite models, I have shown that the presence of the predator population has a clear impact on the potential for host diversity to arise through evolutionary branching. Greater diversity arises in the host population as branching increases with the predation rate for regions where the susceptible and infected hosts coexist with the predator. Here, host populations with different strategies coexist, one

population with high defences and low birth rate and another with low defences and high birth rate (Chapter 2). Additionally, greater host diversity occurs when parasites and predators pose a combined, simultaneous and relatively balanced threat upon the host population (aligning with previous experimental studies). When both enemies are present, coexisting specialist host populations with defence strategies aimed against a particular enemy are likely to evolve as here increasing defence mechanisms against either enemy is beneficial (Chapter 3). It may seem unlikely that the presence of an additional enemy, such as a predator, could increase host diversity, given the addition of increased mortality, but we have seen how the ecological feedbacks between populations can enable such diversity to arise. Clearly the creation of more diversity, relating to the relative abundance and variety of species, is beneficial in nature as it indicates the overall well-being of ecological systems (Magurran, 1988).

In terms of parasite diversity, I found that parasites with different manipulation strategies are not likely to coexist as given the effect of the predation risk on the evolution of host manipulation, this tends to drive the parasites to invest in either low or high levels of manipulation accordingly. As I have shown that host diversity increases where all of these populations coexist, we may have also expected parasite diversity to do the same. However, I have shown that these results are more complex and not always intuitive. Overall, my results have provided vital insight into when diversity is likely to arise in these systems.

It is important that experimental and empirical studies are carried out on host-parasite systems to allow us to test and validate the applicability of theoretical models based on such systems. We can also use them to determine the existence of trade-offs in nature. In the experimental chapter, I aimed to determine whether there is a trade-off or a generalised defence mechanism for the *P. interpunctella* host system against two enemies, PiGV (virus) and *S. aureus* (bacteria). Analysis revealed that the data was non-

significant meaning that the experiment would need to be repeated, possibly with some improvements to the experimental procedure, to gain a clearer insight into the evolution of *P. interpunctella* defence mechanisms against virus and bacteria (Chapter 4). Although I could not make any conclusions from this experiment, given the interdisciplinary nature of this field, it is important that there is continued integration of theoretical work with empirical studies. Specifically, in future experiments, it is vital that population densities are taken into account and recorded as we have seen the importance of population dynamics on evolution.

The spread of infectious diseases through parasites is an interdisciplinary research area of growing interest and importance to mathematicians, biologists, epidemiologists and policy makers as we continue to learn more about the population dynamics, transmission and evolution of the host, as well as the parasite, when an outbreak occurs. My results have added to the development of models for host evolution of defence and parasite evolution of host manipulation within complex communities. Due to the vast, complex range of behaviours and interactions which can arise in nature within host-parasite systems, there are areas that can be studied further as we continue to develop our understanding of this area.

6.2 Future work

My work has given many insights into the evolution of host defence and parasite manipulation when there is a predator present. I have highlighted the importance of the inclusion of a predator as it impacts the evolution of such systems. My work on these models can be taken further as there are many other interesting cases that could also be considered.

I have shown that the addition of the predator population to host-parasite models has a large impact on their evolutionary strategies. Clearly, in nature, more complex situations may arise which could further complicate the

evolutionary behaviour of such systems. By including more populations, we could determine whether our key results still hold or whether they are altered as other population interactions are considered. With the inclusion of another prey species, intraspecific competition between the prey may arise due to common enemies. This may result in the composition of the prey populations, as well as the composition of the enemy community, impacting the evolution of host defence. For example, as discussed in Chapter 2, in a population of two prey species (pink salmon and chum salmon) with a common predator, a generalist parasite (sea lice) lead to a reduction in the predation of chum salmon due to the predators preference for pink salmon (Peacock et al., 2013). This could seem like apparent competition between prey populations due to the predators switching behaviour and could enable the predator population to persist in the system for longer. In this case, our key results may still hold, with the host building up maximum defences where they face the highest combined risk and cost of infection, as we found that using a Holling Type III response (which accounts for prey switching behaviour in the predator) produced similar results (Section 2.3.5). Deeper analysis of this would give more insight.

We could also consider a situation in which the host faces more than two enemy populations. For example, the presence of multiple predators may non-intuitively reduce the risk of predation due to competitive interactions arising between predator populations (Schaller, 1972, Sih et al., 1998). Additionally, the prey may also encounter conflicting defences to multiple predators (Sih et al., 1998). Here, by adding another enemy population, we may find that our key result still holds with the hosts evolution of defence aimed at the most abundant enemy population. Greater host diversity could also arise as the hosts would now have another enemy to defend themselves against creating more specialist host populations with defence aimed at a particular enemy. In terms of parasite evolution of host manipulation, with multiple predators present, the parasites may face a greater cost of evolving

manipulation due to the higher risk of being consumed by a non-suitable predator, or contrastingly, it may become less beneficial for them to evolve manipulation if the additional predators are all suitable final hosts (giving results similar to those in Chapter 5). In this case, the costs and benefits of evolving manipulation may depend on the suitability of the additional predators as final hosts for the parasite. Diversity may also arise in parasite populations as the presence of multiple predators could lead them to specialise into coexisting populations with manipulation strategies aimed at reaching a specific predator species.

We could also consider interactions between different parasite genotypes which could lead to adaptive phenotypic plasticity, where the parasites cooperate if they have closely related genotypes, or competition between genotypes (Leggett et al., 2013). Here, we may see diversity arising in the parasite population or evolution towards a single genotype. This could also produce more diversity in the host as more specialists could evolve with defences against a particular parasite genotype. Therefore, although we have given an insight into host-parasite systems with the inclusion of a predator, our models could be expanded to include more populations to determine how this affects the evolutionary dynamics.

My work has shown the importance of population densities on the evolution of host defence, as well as parasite evolution of host manipulation, however, population densities are often not included in experimental evolution studies. Given the growing body of theory on the importance of ecological feedbacks, and population densities in particular, to evolution, it would clearly be useful to include them in future work, alongside evolutionary trends, as these may give key insights into the drivers of evolution. Such experiments are key to integrate with our theoretical models to show that the results from such work are applicable to real systems.

Our work on the *Plodia interpunctella* host system has shown the difficulties that can arise when carrying out experimental work. This experimental

work could be repeated with improvements (suggestions in Chapter 4) in order to determine the defence mechanisms and any trade-offs used against the virus and bacteria as our data were not reliable enough to make any conclusions. Additionally, experiments could be carried out to determine the parasite costs and mechanisms of manipulation as it is currently not well understood (Poulin, 2010, Maure et al., 2013). Further empirical and experimental studies will give us a better insight into the existence of trade-offs and evolutionary dynamics in host-parasite systems.

Within my work, I have used microparasite models which place the host as either susceptible or infected without explicitly tracking the number of parasites in the population. Contrastingly, we could consider a macroparasite model to look at burden-dependent host manipulation in which the number of parasites a host is infected with is tracked. This may not alter our key results but may lead to cyclical population dynamics as found by Fenton and Rands (2006). In our models, I considered a few different trade-offs which may occur in the host and parasite populations but these could be varied to include trade-offs between different traits, such as a trade-off between host recovery rate and transmission rate, i.e. when the host increases their recovery rate they may constrain their levels of resistance, thereby increasing the transmission rate (Best et al., 2008). It would be interesting to see whether various trade-offs would change our key findings.

I have considered the evolution of the host and parasite populations but we could also consider evolution of the predator population. Particularly in the case where the predator is the final host for the parasite as the predator population may evolve defences against the infection, similar to the hosts defence mechanisms. However, the predator population may not invest in defences as the parasites may not have a sufficiently large enough effect to counter the advantages associated with feeding upon infected prey that are easy to locate and capture (Lafferty, 1992). Furthermore, the coevolution of populations could be considered in our models, for example, the parasite

and predator populations may adapt in response to the hosts evolutionary behaviour to maximise their fitness. Previous work on host-parasite coevolution has considered the parasite evolving in response to the host (van Baalen, 1998, Restif and Koella, 2003, Best et al., 2009, 2010, Boots et al., 2014), with Best et al. (2009) showing that diversity can evolve in both the host and parasite populations. Simultaneous parasite evolution and predator population dynamics have been studied by Kisdi et al. (2013), showing that cyclic dynamics may arise. However, a fully coevolutionary study within complex communities is still to be studied.

The development of this field will assist in maintaining wildlife and managed populations which face risks of infection by diseases transmitted through parasites. The persistence of infections in populations, such as endemic helminth diseases in livestock, is an ongoing issue (Grenfell and Dobson, 1995, Perry and Randolph, 1999). Another major endemic disease is gastrointestinal parasites which many sheep populations face risks of infection from (Nieuwhof and Bishop, 2005). By applying our models to such cases, we could give more beneficial insight on these diseases found in nature. Our results could be used to determine ways to improve conservation as we have shown that the ecological feedbacks between populations can lead to coexistence and increased diversity of species. Such theoretical findings are vital as we have shown that the evolutionary dynamics arising can be non-intuitive. In addition, the costs of treating parasite-transmitted diseases are of key importance to policy makers (Perry and Randolph, 1999, Nieuwhof and Bishop, 2005), hence, our models could be expanded in order to consider the wide economic impact of treating such infections to help find ways of reducing costs. Future work will continue to develop methods for disease control which will assist in maintaining and ensuring the conservation of these populations.

Studying parasitic infectious diseases is vital as they also effect a large number of people, for example, WHO estimates that there were approximately 200 million clinical cases of malaria in 2013 (Baragana et al., 2015).

In the future, our work could be applied to specific diseases such as malaria, as the mosquito acts as an intermediate host carrying the *Plasmodium* parasite which is then passed on to the human host (Churcher et al., 2015). We could build upon our mathematical models of infectious diseases with the use of data from previous and current infectious disease outbreaks or from clinical studies. There is a vast amount of clinical data collected during recent disease outbreaks, such as the Zika and Ebola virus diseases, as well as ongoing endemic neglected tropical diseases, such as helminth diseases, which could be used in future research (Leroy et al., 2004, Cao-Lormeau et al., 2016, Turner et al., 2016).

With these vast possibilities for future work, it would be interesting to see whether these changes would alter our key results or if they would still hold. Clearly, due to the large amount of complexity that can arise in nature, further studies into wider community interactions between hosts and other populations will continue to improve our understanding of the evolution of host-parasite systems within complex communities. As this vital research area continues to attract both theoretical and experimental interest, ongoing work in this area will continue to contribute to furthering and improving our understanding of how to manage and control infectious diseases transmitted through parasites.

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