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The Role of Age-Structure and Genetic Constraints in Host-Parasite Coevolution

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Abstract

Bacteria, viruses and other microparasites can exert strong selection on hosts due to their detrimental effects on fitness. Similarly, host traits are important determinants of the transmission of infectious diseases and consequently parasite evolution. It follows that there is reciprocal evolution in the host and parasite, as each species attempts to maximize its fitness given the ecological interaction. At the centre of the coevolutionary dynamics of the host and parasite there are genetically determined constraints between components of fitness, which determine the course of evolution for each species. Therefore, determining what trade-offs are involved in the interaction and accounting for them are important steps in formulating a coevolutionary theory of hosts and parasites.

This thesis has two distinct foci, a theoretical investigation of host-parasite coevolution (distributed over three chapters) and an experimental test of the costs of host defence against parasite infection (contained within one chapter). The first begins with an examination of parasite evolution in a model accounting for host senescence and is followed by an investigation of how the evolution of senescence depends on parasite infection. We conclude by predicting the coevolution of virulence and host senescence. We then present experimental data regarding how the costs of tolerance to parasite infection are influenced by the environment and sex, using the *Aedes aegypti* mosquito and the microsporidian parasite *Vavria culicis.*

Keywords

Host-parasite interactions, coevolution, trade-off, age-structure, virulence, epidemiology, senescence

Résumé

Les bactéries, les virus et les micro-parasites peuvent exercer une forte sélection sur leurs hôtes en raison de leurs effets négatifs sur leur valeur sélective. De la même façon, les caractéristiques de l'hôte sont des facteurs déterminants de la transmission de maladies infectieuses et par conséquent de l'évolution des parasites. Il s'ensuit une évolution réciproque chez l'hôte et chez le parasite comme chaque espèce tend à maximiser sa valeur sélective en fonction de l'interaction écologique. Au centre de la dynamique coévolutive de l'hôte et du parasite il y a des contraintes génétiquement déterminées entre les composants de la valeur évolutive qui vont déterminer le cours de l'évolution de chaque espèce. Ainsi, en déterminant les compromis évolutifs impliqués dans l'interaction et la façon dont ils influencent son résultat, cela nous permet de comprendre la dynamique coévolutive de l'hôte et du parasite. Ainsi, la détermination des compromis évolutifs impliqués dans l'interaction et de la façon dont ils influencent son résultat, sont des étapes importantes dans la formulation d'une théorie coévolutive entre hôte et parasites.

Ce travail de thèse s'articule autour de deux axes: une investigation théorique de la coévolution hôte-parasite (répartie dans 3 chapitres) et un test expérimental des coûts de la défense de l'hôte contre une infection parasitaire (décrit dans le dernier chapitre). Le premier axe commence par un examen théorique de l'évolution du parasite dans un modèle prenant en compte la sénescence de l'hôte puis est suivi par une investigation de la manière dont l'évolution de la sénescence dépend de l'infection parasitaire. Il s'achève par la prédiction de la coévolution entre la virulence du parasite et la senescence de l'hôte. Ensuite, les données expérimentales sont présentées et démontrent comment les coûts de la tolérance aux infections parasitaires sont influencés par l'environnement et le genre, utilisant le moustique *Aedes aegypti* et le parasite microsporidie *Vavria culicis.*

Mots-clés

Interactions hôte-parasites, coévolution, compromis évolutif, structure par âge, virulence, epidémiologie, senescence

Contents

Abstract 5

Résumé 7

List of Figures 11

List of Tables 12

1. General introduction 13

1.1 Background 13
1.2 Thesis outline 16
1.3 Experimental system 19
1.4 Research aims 21

2. Parasite evolution in an age-structured model 23

2.1 Introduction 25
2.2 Methods 28
2.3 Results 31
2.4 Discussion 34
2.5 Appendix 36

3. Parasites drive the evolution of host senescence 39

3.1 Introduction 41
3.2 The model 43
3.3 Discussion 46

4. Coevolution between parasite virulence and host senescence 49

4.1 Introduction 51
4.2 The model 53
4.3 Results 54
4.4 Discussion 57

5. The impact of food availability and sex on resistance and tolerance of yellow fever mosquitoes against microsporidian infection 61

5.1 Introduction.....63
5.2 Materials and methods.....65
5.3 Results 69
5.4 Discussion.....79
5.5 Appendix.....81

6. Summary and future directions 83

6.1 Summary of results.....83
6.2 Future directions.....84
6.3 Conclusion 86

Acknowledgments 87

References.....89

List of Figures

Figure 1.1 The mosquito life cycle
Figure 1.2 The life cycle of *Vavraia culicis*
Figure 2.1 Survival in the absence of parasite infection
Figure 2.2 Equilibrium values for virulence and prevalence
Figure 2.3 Equilibrium values for the force of infection and average age at Infection
Figure 3.1 Evolved senescence and associated levels of prevalence
Figure 4.1 Evolved virulence and senescence without coevolution
Figure 4.2 Coevolutionary equilibria for virulence and senescence
Figure 4.3 Prevalence at the coevolutionary equilibrium
Figure 5.1 Mean parasite burden and tolerance for larval food, adult reproductive behaviour and sex
Figure 5.2 Correlations between longevity tolerance and mean longevity in the absence of parasites
Figure 5.3 Correlations between longevity tolerance and resistance
Figure 5.4 Correlations of fecundity tolerance with fecundity in the absence of parasites, resistance and longevity tolerance

List of Tables

Table 5.1 Statistical summary for resistance
Table 5.2 Statistical summary for longevity tolerance
Table 5.3 Statistical summary for the role of reproductive behaviour in longevity tolerance
Table 5.4 Statistical summary for fecundity tolerance
Table 5.5 Statistical summary for the role of reproductive behaviour in resistance
Table 5.6 Statistical summary for the ANCOVA for longevity tolerance and longevity in the absence of infection
Table 5.7 Statistical summary for the ANCOVA for longevity tolerance and resistance
Table 5.8 Statistical summary for the ANCOVA for fecundity tolerance and longevity tolerance
Table 5.9 Statistical summary for the ANCOVA for fecundity tolerance and resistance
Table 5.10 Statistical summary for the ANCOVA for fecundity tolerance and fecundity in the absence of infection
Table 5.11 Statistical summary for the post hoc analysis of the role of reproductive behaviour in longevity tolerance

Chapter 1. General introduction

1.1 Background

In underdeveloped areas of the world, parasites are responsible for two thirds of all human deaths (World Health Organisation 2003). The importance of studying how parasites influence host wellbeing is confined not only to the realm of public health, but is also fundamental to how we think about evolutionary processes (Schmid-Hempel 2008). The changes parasites cause in host traits determine not only how hosts interact and reproduce, but also how host genetic diversity is maintained and species diversify. Similarly, hosts influence the evolution of parasites since they ultimately determine parasite survival and transmission (Gandon, Jansen, et al. 2001). Modern epidemiological theory began with the work of Ronald Ross, who developed models to predict the transmission of malaria. A. G. Mckendrick and W. O. Kermack later developed this work, to produce the SIR model. This model accounts for the number of susceptible, infected and recovered hosts in a population. This approach has not only accurately predicted many observed epidemics (e.g. Ferguson et al. 2001), but also forms the basic framework of how we theoretically test ideas about parasite evolution. In the context of this thesis, 'parasite' refers to microparasites, which includes bacteria, viruses, protozoans and fungi.

In a general context, evolution is constrained by genetically determined trade-offs (Loeschcke 1987; Roff 1992; Stearns 1992). For example, an individual can only increase its fecundity by reducing its survival. For parasites, this cost comes in the form of what is known as the virulence transmission trade-off (see Alizon et al. 2009 for a review). The consequence of this trade off is that for a parasite to increase its rate of transmission, it must also increase the mortality of the host. Since a parasite must replicate within a host in order to be transmitted, this replication comes at the cost of increased host mortality. The optimal way for the parasite to balance these two traits is determined by the life history of the host.

When the host has a high risk of mortality from sources other than the parasite, it pays the parasite to be transmitted before the host dies, therefore increasing its virulence (Anderson & May 1982; Gandon, Jansen, et al. 2001; Kakehashi & Yoshinaga 1992).

Similarly, the host pays a cost of reproduction in terms of survival, i.e. increasing fecundity increases age-related increases in mortality rate (senescence). The formal mathematical theory of the evolution of senescence began with the work of W.D Hamilton (Hamilton 1966) and was then refined by Brian Charlesworth (Charlesworth 1994) who demonstrated that senescence could be understood in the context of population genetics theory. Ultimately, Charlesworth showed that in the context of evolution, senescence is a direct consequence of weakening selection pressure with age. Two genetic mechanisms for the evolution of aging are antagonistic pleiotropy (where alleles which are beneficial early in life are deleterious in later life) and the age specificity of gene action (where alleles which are deleterious in later life undergo weak selection). The presence of antagonistic pleiotropy can be tested experimentally by calculating the genetic correlations between life history traits. Negative genetic correlations between longevity and early life fecundity have been found many times in *Drosophila* using a variety of experimental approaches (Rose & Charlesworth 1981; Scheiner et al. 1989; Hughes & Clark 1988) and other genera such as *Poa annua* (Law et al. 2001)and *Caenorhabditis elegans* (Friedman & Johnson 1987). In theoretical studies, the point along this trade-off where the host maximizes its fitness is influenced by the extrinsic mortality risk, i.e. mortality that is not determined by the internal condition of the individual, such as predation or disease. When the extrinsic mortality rate increases, it pays the host to invest heavily in fecundity (at the cost of reduced survival), since it must maximise its reproduction early in life (Williams 1957).

Contemplating the evolutionary costs experienced by the host and parasite and consequently how the evolved traits in one species influence those of the other combines ecological and evolutionary thinking. This approach can be directly applied to agriculture and public health challenges, since the outcome of parasite

evolution, which is determined by host traits, is a central factor determining their capacity to infect new hosts and cause disease. In poultry, shortening of life span and vaccination can increase the virulent effects of Marek's disease virus (Atkins et al. 2013; Rozins & Day 2017) whilst intensive fish farming has resulted in the evolution of highly virulent parasites in which transmission is difficult to control (see Mennerat et al. 2010 for a review). Thinking from evolutionary ecology also suggests ways to control parasite transmission, for example insecticide-resistant mosquitos may pay a cost of lower survival in later life, and are therefore less viable vectors of malaria parasites which develop slowly inside the mosquito (Koella et al. 2009).

It follows naturally from thinking about evolution in either the parasite or host, that there is likely to be on-going evolution, as either species evolves, the other must adapt its life history strategies to maximize its fitness. Although Darwin was the first to consider how the reciprocal changes in the traits of two interacting organisms may alter the course and rate of long-term evolution, it took over 100 years before the term *coevolution* was coined by Paul Ehrlich and Peter Raven in an attempted to understand the evolutionary interactions of plants and butterflies (Ehrlich & Raven 1964). More recently, studies have investigated the coevolution of host and parasite life histories (Koella *et al.* 2001, Gandon 2002, Ashby & Boots 2014). As we have seen, the evolution of both the host and parasite is constrained by genetically determined trade-offs. These trade-offs are therefore at the centre of the coevolutionary dynamics of the host and parasite.

Through altering its life history, the host can mitigate the damaging effects of parasitism. For example *Biomphalaria glabrata* snails mature earlier when exposed to the sterilising trematode parasite *Schistosoma mansoni* (Minchella & Loverde 1981), whilst *Cerithidea californica* snails evolve to mature as smaller individuals in areas with high parasite prevalence (Lafferty 1993). This finding also receives theoretical support, which suggests that infected individuals will evolve to mature and reproduce earlier than uninfected ones (Hochberg et al. 1992). There are however more direct ways by which a host can reduce the

impact of parasite infection on its fitness. For example, a host may evolve physiological processes to reduce parasite growth (*resistance*) or to reduce the damaging effects of parasite growth (*tolerance*) (Råberg et al. 2009). Differentiating between these types of host defence complicates the outcome of coevolution since the two tactics have different implications for parasite fitness (Roy and Kirchner 2000; Miller et al. 2006). Tolerance has a positive or neutral outcome for the parasite, whilst resistance has negative implications for the parasite since it can reduce transmission. The cost of these defence strategies is likely to be a strong determinant of host evolution and consequently the optimal strategies of the parasite. If resistance and tolerance to parasite infection are costly in terms of other host life history traits, their maintenance within populations is likely to be determined by the incidence of parasite infestation in the environment. In the absence of parasites, selection may drive defence traits to low levels if they are negatively correlated with beneficial host life history traits. Furthermore, it is well known that the genetically determined correlations between life history traits can change depending on the environment (Sgro & Hoffman, 2004). A dependency of the costs of host defence against parasites on the environment is likely to have important implications for host-parasite coevolution, since it is inevitable that individuals experience variation in biotic and abiotic components of the environment.

1.2 Thesis outline

This thesis uses both theoretical and empirical approaches to develop our understanding of host-parasite coevolution. It begins by developing epidemiological theory to allow the calculation of parasite evolution whilst explicitly accounting for host senescence, an aspect that is currently lacking in the literature. Through this formulation, we highlight several aspects of host-parasite dynamics that have added importance in an age-structured setting, compared to the classical age-independent setting, which is most frequently used for predicting parasite evolution. We use this framework to test how parasite virulence influences the evolution of host senescence and then implement a

coevolutionary theory of virulence and host senescence. Finally, we present experimental work on the genetically determined costs of host defence to parasite infection and how they are influenced by environment and sex.

1.2.1 An age-structured theory for predicting parasite evolution

An age-dependent increase in mortality rate (senescence) is found in many organisms and has been a large focus of evolutionary thinking in previous years. However, this component of host life history has remained absent in theoretical studies of parasite evolution. To address this issue, we develop an existing age-structured theory of epidemiology (Anderson & May, 1983) to allow the calculation of parasite evolution. In simple epidemiological models, increases in the background mortality of the host selects for increased parasite virulence (Anderson & May 1982). When the host's background mortality rate increases with age, it follows that the age at infection of the parasite is likely to influence the equilibrium virulence. We investigate which parameters influence the average age at infection and consequently what outcome they have for parasite evolution.

1.2.2 Senescence evolution in the context of epidemiological dynamics

Although the evolution of senescence in response to extrinsic mortality has received frequent theoretical attention (Williams 1957; Medawar 1952; Williams & Day 2001; Abrams 1993; Shokhirev & Johnson 2014), how parasite infection and transmission determine optimal levels of senescence is still unknown. Classical models predict higher levels of extrinsic mortality should select for higher senescence, although models accounting for ecological interactions can produce contrasting predictions. Given that the host-parasite interaction determines parasite transmission and therefore the prevalence, how parasite-induced mortality should influence the evolution of senescence is unclear. In the third chapter, we investigate how parasite virulence shapes the evolution of

senescence. We test how levels of presenescent mortality and variations in the trade-off between senescence and fecundity influence this relationship.

1.2.3 A coevolutionary theory of parasite virulence and host senescence

Our understanding of the coevolution of host and parasite life histories is well developed (Koella & Restif 2001, Gandon *et al.* 2002, Ashby & Boots, 2015), yet a theory accounting for host senescence is lacking. The approach developed in the second and third chapters of the thesis is extended to allow for the calculation of the coevolutionary equilibrium. We test how aspects of host life history, and parasite transmission influence the predictions of the model and the capacity for parasite-driven host extinction. We show that taking account of the coevolutionary dynamics changes where in the parameter space host extinction occurs.

1.2.4 The costs of host defence against parasite infection

It is widely acknowledged that host defence to parasite infection can not only involve the restriction of parasite growth (resistance), but also the ability to control the damaging effect of a given parasite burden (tolerance). Theoretical studies suggest that distinguishing between these defence strategies can have very different outcomes for host-parasite coevolution (Roy & Kirchner 2000; Best et al. 2008; Restif & Koella 2003a). Although several studies have investigated the costs of the defence strategies in relation to each other, evidence of the costs of tolerance in relation to other host life history traits is rare in animal systems. Consequently, it is also relatively unknown what role the environment plays in these costs. In nature, individuals can experience changing environmental conditions. If a host's environment determines the costs of parasite defence, environmental fluctuations will influence the evolutionary outcomes of host-parasite interactions. Indeed, the genetically determined correlations between life history traits can change depending on the

environment (Sgro & Hoffman, 2004). In the fifth chapter, we test the influence of host sex and food availability on the costs of tolerance and resistance.

1.3 Experimental system

To test for the genetically determined costs of resistance and tolerance, we used the mosquito *Aedes aegypti* (from Patrick Guérin, University of Neuchâtel) and the microsporidian parasite *Vavraia culicis* (from James Becnel, USDA, Gainesville, USA). *Aedes aegypti* is a vector of clinically important diseases, such as Zika virus, dengue and yellow fever. It occurs through the tropics and sub tropics, and can lay eggs in both naturally occurring formations of water or artificial containers (Southwood 1975, see Fig. 1.1 for its life cycle). Its ecology has been well studied (Christophers 1960) and it is widely used in insect physiology experiments (Clements 1999).

Vavraia culicis is an obligate unicellular fungal parasite, which infects several mosquito genera including *Aedes* and *Anopheles*. The parasite infects an individual through gut epithelial cells after ingestion. Although there is no trans ovarian transmission, spores can be laid on eggs (Andreadis 2007) and subsequently infect the newly hatched larvae. Under particular environmental conditions, infection can kill individuals during the larval stage (Bedhomme *et al.* 2004), which releases spores into the aquatic environment and then begins another round of horizontal transmission (Fig. 1.2).

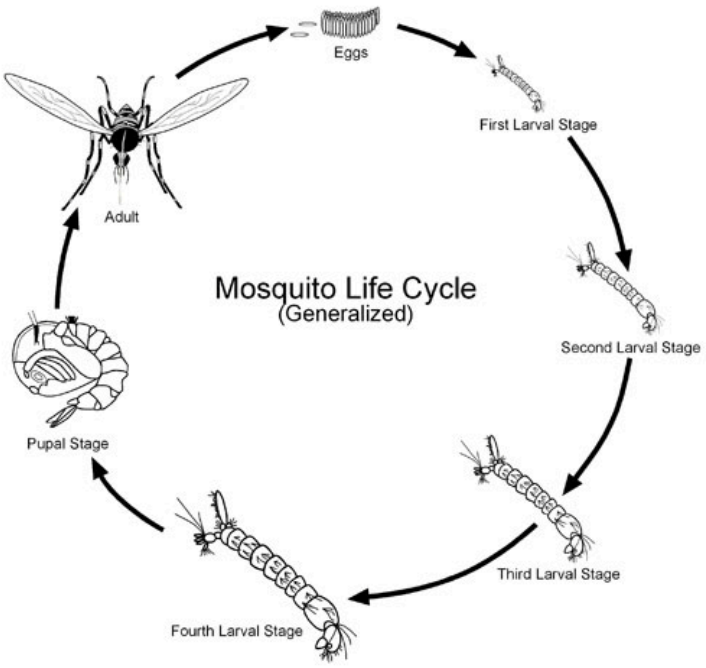

Figure 1.1: The mosquito life cycle

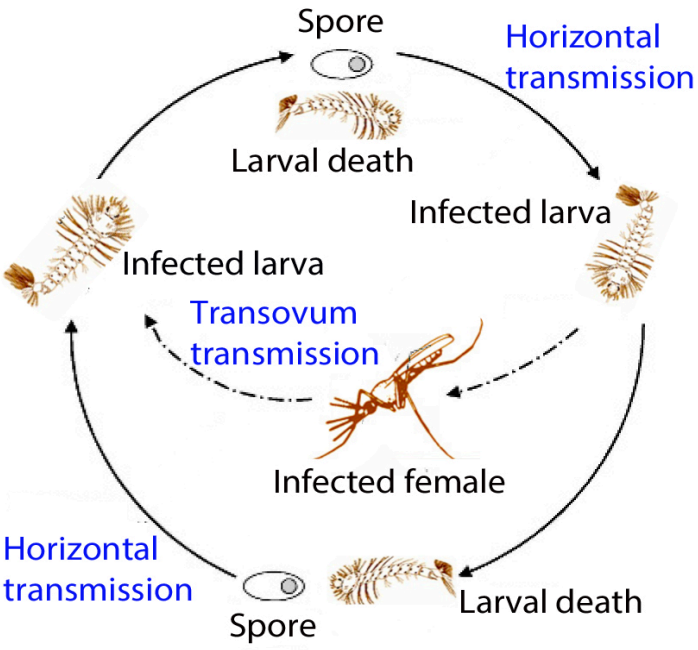

Figure 1.2: The life cycle of the microsporidian parasite *Vavraia culicis*

1.4 Research aims

The goal of this thesis is to develop our understanding of host-parasite coevolution in two ways. Firstly we add to the current theory by accounting for host age-structure, which ultimately allows us to include a trade-off between host senescence and fecundity. This trade-off forms the basis of the evolutionary theory of senescence, but is lacking in models of host-parasite interactions. Then, we move away from determining the implications of genetic constraints, to testing what constraints may be involved in host defence against infection. Understanding these constraints will aid us in interpreting the predictions of coevolutionary models of parasite traits and host defence, since they allow us to assess the accuracy of model assumptions.

Thus, the work herein can be divided as follows:

I. Extend an existing age-structured epidemiological model with parasite evolution.
II. Determine how parasite infection drives the evolution of host senescence.
III. Calculate the coevolutionary equilibrium for virulence and host senescence.
IV. Test experimentally for the costs of tolerance and resistance against parasite infection, and determine if they are influenced by sex and the environment.

Chapter 2. Parasite evolution in an age-structured model

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Abstract

The influence of host life history traits on the evolution of parasite virulence is discussed in many experimental and theoretical studies. Nevertheless, increasing mortality rate with age (senescence), which is an important life history character of many organisms, is missing from models of parasite evolution. Given that the host's mortality rate influences virulence evolution, the average age at infection is likely to determine the equilibrium virulence when senescence is accounted for. Since, then, virulence influences transmission and thus the average age at infection, we expect epi-evolutionary feedbacks underlying the evolution of virulence in a senescing population. We evaluate this idea by extending an existing age-structured epidemiological model with the parasite's evolution. The model predicts that increasing the strength of senescence forces the evolution of higher virulence. Additionally, we show that the choice of transmission function and host population growth parameters influence the average age at infection and consequently the equilibrium virulence. Our results demonstrate that incorrect specification of the transmission function may give misleading predictions for virulence evolution when senescence constitutes part of the biological system in question.

2.1 Introduction

The application of epidemiological models in an evolutionary context has provided major insights into how vaccination and host traits, such as behaviour and mortality, shape the evolution of virulence (Gandon, Mackinnon, et al. 2001; Ashby & Boots 2015; Gandon & Michalakis 2000; Gandon, Jansen, et al. 2001). Central assumptions of these studies are that the transmission of the parasite is related to its density within the host, and that rapid replication of the parasite within the host increases the host's mortality rate (virulence), leading to a trade-off between transmission and mortality rates. The parasite can therefore replicate rapidly, increasing short-term transmission, but reducing future opportunities to infect new hosts, or can replicate slowly to increase the likelihood of new infections in the more distant future (Alizon et al. 2009; Ewald 1983; Anderson & May 1982). It follows that the optimal virulence is dependent on the background mortality of the host (the mortality rate due to sources other than parasitism). If the background mortality of the host increases, a parasite can maximize its total transmission by increasing its current transmission at the expense of future transmission.

Although the importance of host life history traits in this trade-off and in parasite evolution is well established (Gandon, Jansen, et al. 2001; Koella & Restif 2001; Gandon et al. 2002), what is missing from theoretical studies is that the rate of background mortality of many hosts increases with age, that is that the hosts senesce. To account for host senescence in parasite evolution, an age-structured theory is required. Although these models are well developed and have been applied to clinical diseases (Anderson & May 1983), they are yet to be used in the context of virulence evolution.

It follows, that the age at which the host becomes infected is likely to influence the equilibrium virulence. Consider a scenario where the host is infected at a young age; here the individual has a low background mortality rate and it therefore pays the parasite to be prudent with its level of virulence, since it will benefit from an increase in future transmission possibilities. If we then consider

an old host, one with a higher background mortality rate relative to its younger counterparts, the parasite must maximize its chance of transmission in a shorter period of time, which should result in a higher level of virulence increasing its fitness. We have seen only one discussion related to this topic, which suggests that parasites may exhibit plasticity for transmission mode (either vertical or horizontal), with parasites demonstrating horizontal transmission when they infect older individuals (Kysela & Turner *unpublished*, see also Koella & Turner (2008) for a discussion).

The average age at which individuals become infected is determined by the so called 'force of infection', which can be formulated in terms of the number of infected individuals (resulting in *density-dependent transmission*) or the proportion of individuals in the host population which are infected (resulting in *frequency-dependent transmission*). Assuming X and Y represent the number of susceptible and infected individuals, whilst N and β are the total population size and the transmission coefficient, the force of infection is then given by either $\lambda = \beta XY$ (density-dependent transmission) or $\lambda = \beta XY/N$ (frequency-dependent transmission). The formulation of the transmission function has been widely debated (de Jong et al. 1995; Begon et al. 2002; McCallum et al. 2001) and there is an extensive discourse about how different host parasite systems show a tendency for one type of transmission over the other, or sometimes a combination of the two (McCallum et al. 2001). For example, in sexually transmitted infections and vector-borne diseases, it is reasoned that even at a low population density, individuals will seek out others, rather than randomly encountering them (Thrall et al. 1993; Lockhart et al. 1996). Transmission of the parasites causing these diseases is therefore more accurately described as frequency-dependent process. What is clear from theoretical studies is that distinguishing between the two transmission functions influences our intuition concerning parasite and host extinction. When transmission is density-dependent, the parasite cannot cause host extinction, for once the host density is reduced below a certain threshold, the parasite population will go extinct. Indeed one motivation for modelling transmission as a density-dependent process is that it enables the calculation of the minimum host population size for

parasite establishment (Anderson & May 1979; May & Anderson 1979; Anderson & May 1978; May & Anderson 1978). Under frequency-dependent transmission, this threshold does not exist and a host population can be driven to extinction by the parasite (Getz & Pickering 1983, Boots & Sasaki 2003). However, assuming a parasite can invade a population, the use of frequency or density-dependent transmission has no influence on the equilibrium parasite virulence in current models (O'Keefe 2005), which do not include age-dependent mortality. One motivation for our study is to test whether this distinction between frequency and density-dependent transmission holds when host senescence may interact with the choice of transmission function to influence the average age at infection.

Although there is one study that shows the carrying capacity of the host can influence virulence evolution when host mortality is due to a dynamical predator (Morozov Yu. & Adamson 2011), simpler models show no dependency of virulence evolution on the growth parameters of the host population, assuming the parasite is endemic. Since these parameters determine the input of new susceptible individuals into the host population, how they influence transmission (and the average age at infection), will depend on the formulation of the transmission function.

The principle goal of this article is to test how host senescence influences the evolution of virulence, and whether this depends on the average age at infection. Subsequently, we investigate how host population growth and the transmission function determine the average age at infection, and therefore what importance they have in age-structured models of parasite evolution.

2.2 Method

Following Anderson & May 1983, we assume that there is no recovery of infected individuals and therefore describe the epidemiology of the system with:

$$\frac{\partial X}{\partial t} + \frac{\partial X}{\partial a} = -(\lambda(t) + \mu(a))X(a,t)$$

$$\frac{\partial Y}{\partial t} + \frac{\partial Y}{\partial a} = \lambda(t)X(a,t) - (\alpha + \mu(a))Y(a,t)$$

where $X(a,t)$ and $Y(a,t)$ are the number of susceptible and infected individuals at age a and time t, α is the virulence (the parasite-induced mortality of the host), $\lambda(t)$ is the force of infection at time t, and $\mu(a)$ is the mortality rate at age a. The boundary conditions are:

$$X(0,t) = \int_0^\infty b(X(a,t) + Y(a,t))\, da$$

$$Y(0,t) = 0$$

where b is the birth rate. The number of infected and susceptible individuals across all age groups at equilibrium is given by setting the derivative with respect to t to 0:

$$\frac{dX}{da} = -\big(\lambda + \mu(a)\big)X(a)$$

$$\frac{dY}{da} = \lambda X - \big(\alpha + \mu(a)\big)Y(a)$$

We introduce an age dependent mortality rate, $\mu(a)$, given by:

$$\mu(a) = \mu_0 + \kappa a^n$$

With this mortality function, it follows that the probability of surviving up to age a, $l(a)$, in the absence of parasite infection is given by:

$$l(a) = e^{-\mu_0 a - \frac{\kappa a^{n+1}}{n+1}}$$

We then see that n controls how the probability of survival decreases with age (Fig. 2.1). Note that when n =0, we set κ=0.

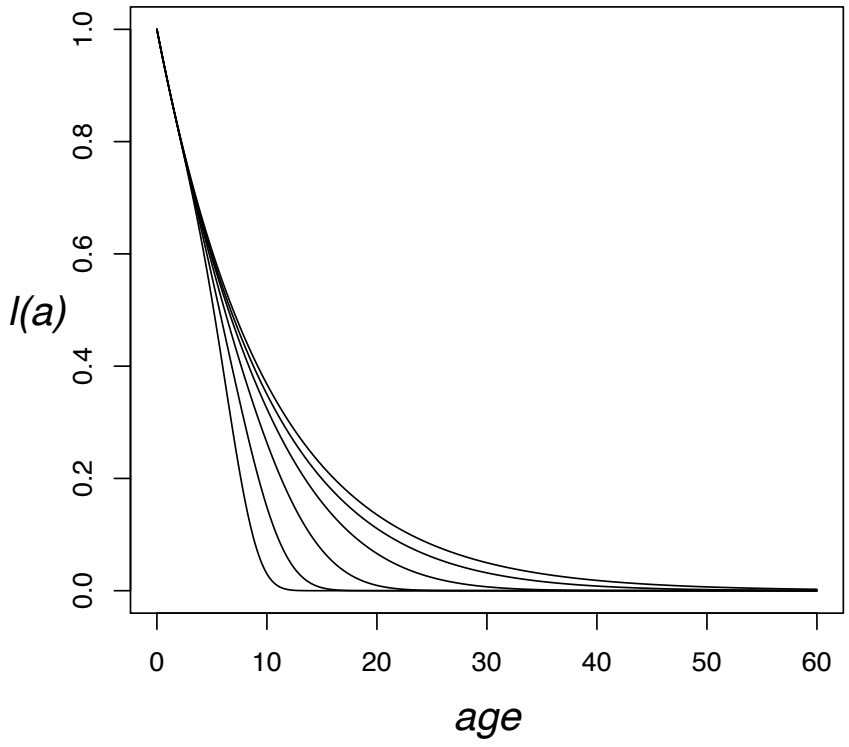

Figure 2.1: Survival curves in the absence of parasite infection for n=0 (far right), 1, 1.5, 2, 2.5 (far left). Increasing n reduces survival. μ_o=0.1 and κ = 0.001

Assuming a virulence-transmission trade off, we set the coefficient of transmission to $\beta = \beta_0\sqrt{\alpha}$. The force of transmission is modeled in terms of either the proportion or number of infected individuals:

$$\lambda = \beta\, \bar{Y}/\bar{N}$$

$$\lambda = \beta\, \bar{Y}$$

Where $\bar{N} = \bar{X} + \bar{Y}, \bar{X} = \int_0^\infty X(a)\, da, \bar{Y} = \int_0^\infty Y(a)\, da$, and all values are obtained at the epidemiological equilibrium. To obtain the equilibrium, we iterate cohorts assuming $Y(0) = 0$, whilst updating $X(0)$ and λ. We assume density-dependent population growth and that infection does not alter the birth rate, such that:

$$X(0)_{c+1} = \bar{N}_c b_0\, e^{-\nu \bar{N}_c}$$

where subscript c is the cohort and ν is calculated from a carrying capacity K with:

$$\nu = \frac{\ln(b_0 L)}{K}$$

Where L is the life expectancy of the host:

$$L = \int_0^\infty \frac{X(a) + Y(a)}{X(0)}\, da$$

The average age at infection is given by:

$$A = \frac{\int_0^\infty a\lambda\, X(a) da}{\int_0^\infty \lambda\, X(a) da}$$

Increasing the force of infection tends to decrease the average age at infection (Anderson & May, 1991). Although a robust approximation for the basic

reproductive number is given by the reciprocal of the proportion of susceptible individuals at equilibrium, it is not clear whether this is an accurate measure of parasite fitness in a model including host senescence. Consequently, we use evolutionary invasion analysis with competition between two parasite strains to calculate the equilibrium virulence (see Appendix). We test the influence of n on the equilibrium virulence for both transmission functions over a range of values for K and b_0, and then assess the role of the population growth parameters in determining the average age at infection and the force of infection. We do not explore the entire parameter space for the models, but a range of parameters to indicate the possible evolutionary outcomes.

2.3 Results

For both density and frequency-dependent transmission increasing the strength of senescence (n) increases the virulence (Fig.2.2a and 2.2b) and decreases the prevalence (Fig. 2.2c and 2.2d). When there is no host senescence ($n = 0$), we retrieve the prediction of classical epidemiological models: that host population growth and the choice of transmission function do not influence the equilibrium virulence. When host senescence is accounted for, the choice of transmission function determines whether the parameters of host population growth influence virulence evolution.

For frequency-dependent transmission, the host's birth rate and carrying capacity have no influence on the equilibrium parasite virulence. However, when transmission is density-dependent, increasing the birth rate or carrying capacity results in the evolution of lower virulence and higher prevalence. The influence of the host population growth parameters increases with the strength of senescence; for low values of n we see far smaller differences between the birth rates and carrying capacities than when n is large. These results can be understood in terms of the equilibrium values of the force of infection and average age at infection, which are both influenced by the parameters of host population growth when transmission is density-dependent (Fig. 2.3). For

frequency-dependent transmission, as long as the birth rate and carrying capacity are high enough for the host population to persist, the same equilibrium for the force of infection and average age at infection will be reached regardless of the host population growth parameters.

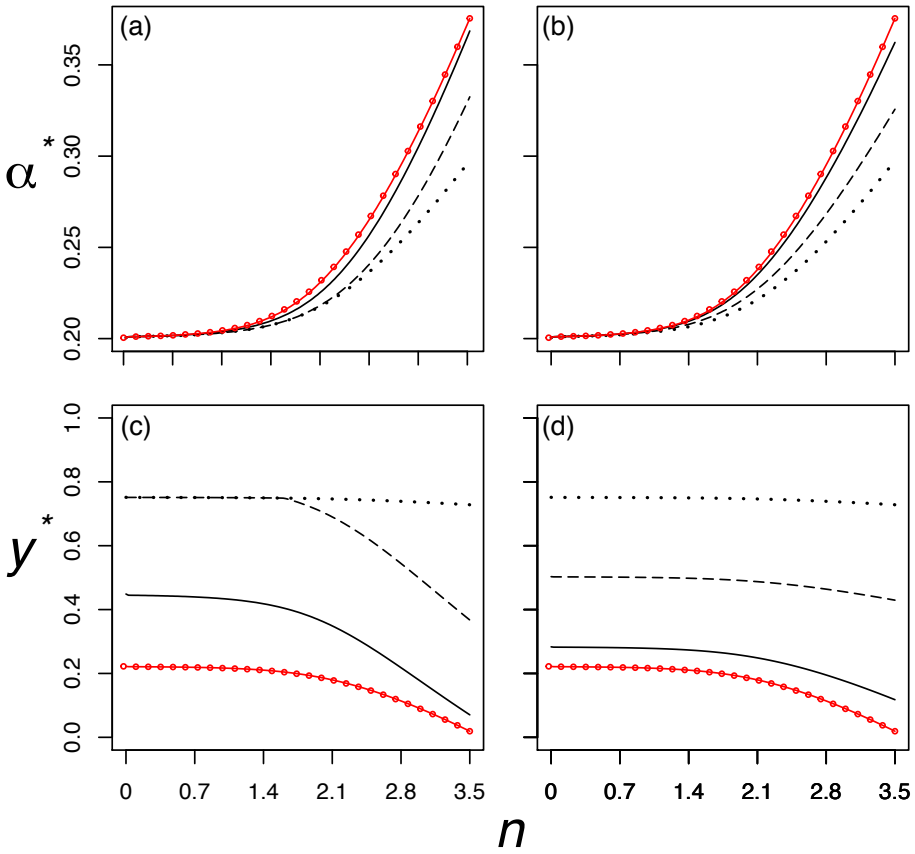

Figure 2.2: Equilibrium values for virulence and prevalence (y^*), for different values of n, b_0 (plots a and c), and K (plots b and d) for frequency (red) and density (black) dependent transmission. For density-dependent transmission b_0=0.29 (solid), 0.36 (dashed), 0.6 (dotted) and K=1040 (solid), 1500 (dashed), 3000 (dotted). For frequency - dependent transmission b_0=0.29 (solid) and 0.6 (circles), K=1040 (solid) and 3000 (circles). $X(0)$ = 500 (starting condition), λ=0.01 (starting condition), μ_0=0.2, κ=0.0005, $\beta_0 = 0.0012$ (density-dependent transmission) and 1.15 (frequency-dependent transmission)

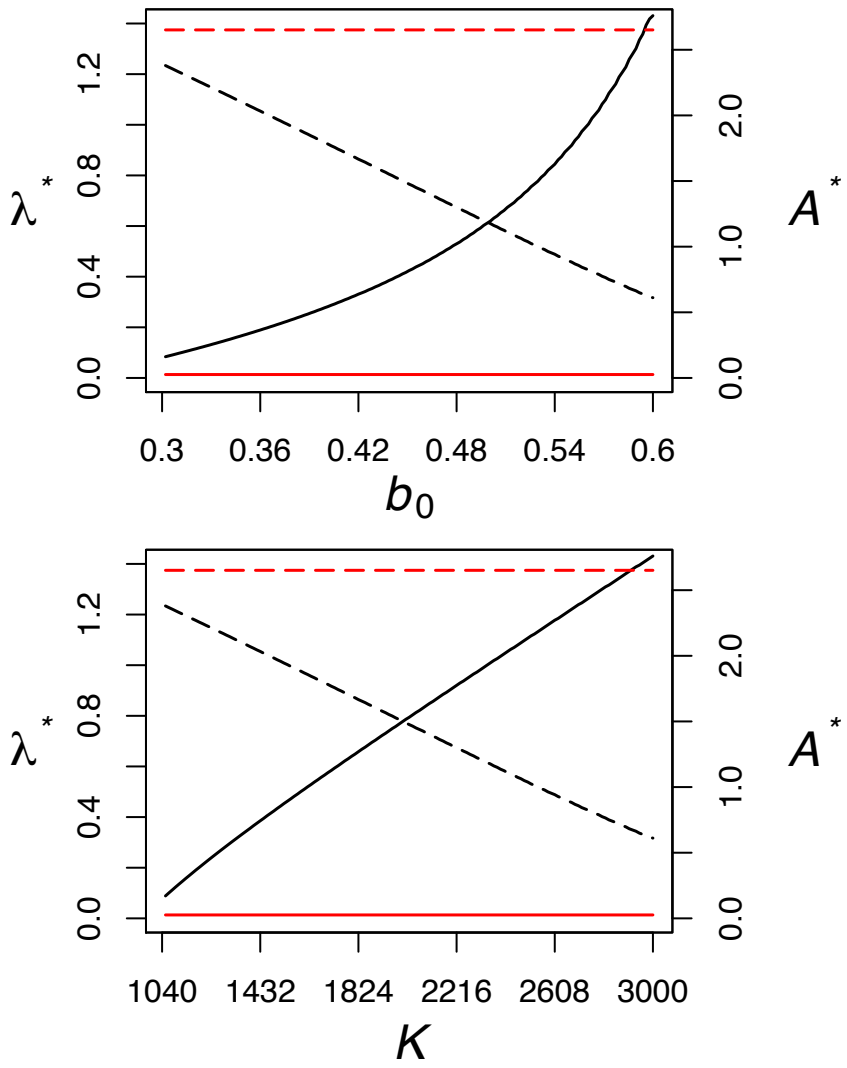

Figure 2.3: Equilibrium values for force of infection (solid lines) and average age at infection (dashed lines) for increasing values of birth rate (top panel) and carrying capacity (bottom panel). The host population growth parameters influence the force of infection and average age at infection (A^*) when transmission is density-dependent (black) but not when it is frequency-dependent (red). For the top panel K= 3000 and for the bottom b_0= 0.45. $X(0)$ = 500 (starting condition), λ=0.01 (starting condition), μ_0=0.2, κ=0.0005, $n = 3.5$, $\beta_0 =$ 0.0012 (density-dependent transmission) and 1.15 (frequency-dependent transmission)

2.4 Discussion

Our results suggest that the strength of host senescence is an important determinant of virulence evolution. When host senescence is accounted for, the way parasite transmission is formulated can influence the dependence of the equilibrium virulence on host population growth. These findings are a consequence of the birth rate and carrying capacity influencing the average age of infection when transmission is density-dependent, but not when it is frequency-dependent. The framework we present is important not only in its own right, since senescence is an important component of the biology of many organisms, but also because it adds a further layer of complexity to the problem of how parasite transmission should be modelled. Although it is known that the choice of transmission function determines whether a parasite can force the host population to become extinct (Boots & Sasaki 2003), this is the first demonstration that it can also influence the evolution of virulence.

In this study and the majority of those on parasite evolution (see Cressler et al. 2015 for a review), virulence is considered in the context of host mortality. Reductions in host mortality influence the fitness of the parasite, since they allow less time for it to be transmitted before the host dies. In age-independent models, when parasite transmission is assumed to be costly in terms of host fecundity (sterility virulence), the parasite will evolve to completely sterilize its host, since lower host fecundity does not (in itself) influence parasite fitness (O'Keefe & Antonovics 2002; Jaenike 1996). We show that the fecundity of the host determines the average age at infection under density-dependent transmission. If we consider this result in the context of a sterility virulence-transmission trade-off and host senescence, the parasite may not necessarily always evolve to sterilize the host. Take a highly virulent parasite that causes low host fecundity. This parasite will only infect old individuals, which have a high background mortality rate. A parasite with low virulence will allow hosts a high birth rate, resulting in a low average age at infection, at which infected individuals will have a low background mortality rate. Thus, the birth rate will influence the fitness of the parasite in the age-structured setting, since it changes

the background mortality rate of the host it infects. This makes the evolutionary outcome for sterility tolerance less clear than in the standard model, in which mortality rate is constant with age.

The primary limitation of the work here is that we simplify transmission into two extremes; it is either frequency or density-dependent. Transmission may occur via a combination of frequency and density dependent transmission, which can have implications for parasite driven extinction and thresholds of parasite persistence (Ryder et al. 2007). Transmission may also have a non linear relationship with host density (Knell et al. 1996), which can have important implications for the dynamics of the host-parasite system (Hochberg 1991).

Notice should also be given to the implicit assumptions about the area in which individuals interact. Here, we assume that this area is constant, such that for density-dependent transmission, increasing the birth rate and thus the number of susceptible hosts, increases the contact rate between individuals (Begon et al. 2002). The validity of this assumption varies depending on the biology of the system in question. As pointed out by Begon et al. (2002), studies on field populations most likely assume a fixed area, since sampling usually takes place in the same area, regardless of the number of individuals sampled. In contrast, domestic animals are likely to occupy larger areas as the number of individuals in the population increases. Under frequency-dependent transmission, although the area is also constant, each susceptible host makes the same number of contacts with each infected one, regardless of the input of new susceptible individuals. As a result, the birth rate and carrying capacity of the host do not influence the force of infection. Under density-dependent transmission, since increasing the number of individuals in a given area increases the incidence of contact, this will increase the number of infected individuals (and therefore the force of infection).

In summary, this work suggests that taking account of host senescence changes our current understanding of how the transmission function and host population growth influence virulence evolution. Simplifying transmission to a density

dependent process as a first approximation should be approached with caution, since it may give misleading predictions regarding parasite evolution in an age-structured setting.

2.5 Appendix

Parasite evolution

To calculate the optimal parasite virulence we use evolutionary invasion analysis, taking into account epidemiological feedbacks. This implies that the mutant strain directly influences host population growth and the force of infection of the resident. To begin, we extend the basic model to include two strains:

$$\frac{dX}{da} = -\big(\lambda_r + \lambda_i + \mu(a)\big)X(a)$$

$$\frac{dY_r}{da} = \lambda_r X - \big(\alpha_r + \mu(a)\big)Y_r(a)$$

$$\frac{dY_m}{da} = \lambda_m X - \big(\alpha_m + \mu(a)\big)Y_m(a)$$

Where the subscript r denotes the resident strain and m denotes the mutant.
The initial force of infection for the mutant is calculated assuming the mutant infects a small proportion of the hosts infected by the resident strain (which are subsequently removed from the part of the host population infected by the resident).

To calculate the equilibrium virulence we first simulate the resident population to equilibrium, in the absence of the mutant. Then we set the mutant's virulence to a value just below that of the resident. We search for the resident α at which the growth rate of the mutant changes sign. This gives the point at which the two

isoclines intersect, when the outcomes of invasion for a range of combinations of α for the resident and mutant are plotted.

We iterate cohorts in the same manner as was done to obtain the equilibrium conditions of the resident. We use a density dependent birth rate to obtain $X(0)$ for each cohort, assuming both uninfected individuals and those infected by either the resident or mutant reproduce.

The force of infection for each of the two strains, for frequency-dependent transmission, is given by:

$$\lambda_r = \beta_r \bar{Y}_r / \bar{N}$$

$$\lambda_m = \beta_m \bar{Y}_m / \bar{N}$$

Where:

$$\beta_r = \beta_0 \sqrt{\alpha_r}$$

$$\beta_m = \beta_0 \sqrt{\alpha_m}$$

$$\bar{Y}_r = \int_0^\infty Y_r(a)\, da$$

$$\bar{Y}_m = \int_0^\infty Y_m(a)\, da$$

$$\bar{N} = \int_0^\infty X(a) + Y_r(a) + Y_m(a) da$$

When transmission is density-dependent the force of transmission is given by:

$$\lambda_r = \beta_r \bar{Y}_r$$

$$\lambda_m = \beta_m \bar{Y}_m$$

To calculate the growth rate of the mutant, we simulate cohorts and then take the logarithm of the slope from the linear model of iteration against $\bar{Y}_m/\bar{Y}_r$.

To assess the validity of this approach, we verified that the equilibrium values given for constant mortality where the same as those given when maximizing R_0 from the age independent model ($R_0 = \frac{\beta K}{\alpha + \mu}$, where K is the disease free equilibrium population size). We found that to retrieve these predictions several iterations of the invasion were required before calculating the growth rate of the invading strain.

Chapter 3. Parasites drive the evolution of host senescence

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Abstract

Senescence, the age-related increase in mortality rate, has been a central topic in evolutionary thinking for many years. A prediction of the evolutionary theory of senescence is that externally imposed mortality should force the evolution of increased senescence. More complicated models, for example, which account for ecological interactions, can predict reduced senescence in response to increasing external sources of mortality. Although parasitism is an important force in the evolution of host life histories, its role in shaping senescence remains untested. Here we calculate age-specific host survival, whilst accounting for the epidemiological dynamics of a parasite. We find that increasing parasite-induced mortality selects for higher levels of senescence, and that this is consistent for a range of presenescent mortality values. Our results therefore suggest that accounting for host-parasite interactions should not necessarily result in variations of the predictions of the classical theory of senescence evolution.

3.1 Introduction

Since the work of Medawar (1952) and Williams (1957), the idea that higher levels of mortality from external factors (extrinsic mortality) should force the evolution of higher senescence (increasing mortality rate with age) has been the focus of ongoing investigation. Williams' evolutionary theory of senescence is based on the idea that increases in fecundity should come at the cost of a decrease in longevity (due to antagonistic pleiotropy). When this trade-off is present, an increase in extrinsic mortality should force the evolution of higher reproduction at early ages, resulting in higher senescence. Theory has since predicted that this may not be the case in all biological settings. For example, extrinsic mortality can increase or decrease the equilibrium senescence depending on assumptions about the density-dependent processes involved in population growth (Abrams 1993) or interactions between sources of extrinsic mortality and the internal condition of the individual (Williams & Day 2001). Additionally, individual-based models suggest that increasing predation can lower the optimal senescence, for certain costs of mating and food availabilities (Shokhirev & Johnson 2014). Experimental evidence on the role of extrinsic mortality in senescence evolution is mixed. In fish, extrinsic mortality can select for earlier maturity, although this was associated with a reduction in swimming performance with age rather than in mortality or fecundity (Reznick et al. 2004). However, *Drosophila melanogaster* individuals experiencing lower extrinsic mortality were successfully selected to have lower intrinsic mortality (Stearns et al. 2000), and populations of *Daphnia* exposed to low levels of external mortality showed lower rates of intrinsic mortality than those in environments characterized by high extrinsic mortality (Dudychai & Tessier 1999). Clearly, ecological circumstance and species-specific differences in life history can play important roles in the evolution of senescence.

Parasites can be an important source of mortality for their hosts in natural systems, yet there is no theory of their role in the evolution of senescence. A frequent assumption in models of parasite epidemiology is that increasing transmission comes at the cost of increasing the mortality of the host. Whether

this parasite-induced mortality (virulence) should have simple outcomes for senescence evolution is unclear, since it also alters the prevalence of infection in the host population. When epidemiological dynamics are accounted for, increasing virulence should reduce the prevalence of infection in the host population (Anderson & May, 1978). Similarly, increasing the mortality rate from sources other than parasitism for a given virulence should also decrease the prevalence, since it reduces the time available for parasite transmission. The presenescent mortality rate can be considered as a constant form of extrinsic mortality. If the mortality rate of the host at age a is given by $\mu(a) = \mu_0 + \kappa a^n$ then the presenesent mortality rate is given by μ_0, whilst κa^n determines the increase in mortality as a function of age (Chapter 2). Without parasites, increasing the presenescent mortality rate should force the evolution of higher senescence, since it decreases the number of deaths due to increasing mortality rate with age (Williams 1957; Rose 1991; Ricklefs 1998). When presenescent mortality is low, more individuals are exposed to selective forces later in life, resulting in the evolution of delayed senescence. In this study, we take account of two sources of extrinsic mortality: virulence, which can reduce the survival of many or few of the individuals depending on epidemiological dynamics, and another constant source μ_0 which affects all individuals in the population.

The combined impact of presenescent mortality and parasite virulence in determining the equilibrium senescence is unclear. For example, high presenescent mortality for a given virulence should result in low prevalence, which may then result in the evolution of lower senescence than when presenescent mortality is low, since there are fewer deaths in the population due to parasitism. Whether this is the case will depend on the functional relationship between presenescent mortality and prevalence, which will in turn be influenced by the strength of senescence and virulence.

Using an age-structured epidemiological model, we test how parasite virulence influences the evolution of senescence for a range of values for the presenescent mortality rate, as well as variations in the trade-off between senescence and fecundity.

3.2 The model

We obtain the epidemiological equilibrium as in chapter 2, by iterating cohorts and taking account of the density-dependent birth rate, with the exception that the birth rate in the absence of density-dependent effects depends of the level of senescence:

$$X(0)_{c+1} = \overline{N}_c b\, e^{-\nu \overline{N}_c}$$

We assume a trade-off between the birth rate, b, and the level of senescence:

$$b = b_0 + sn^{\eta}$$

where the birth rate in the absence of senescence is given by b_0 and the shape of the trade-off is determined by η. Note here, that b_0 has a different interpretation than in chapter 2. As before, the mortality rate at a given age is:

$$\mu(a) = \mu_0 + \kappa a^n$$

It follows that the rate of senescence is given by:

$$\phi = \kappa^{\frac{1}{n+1}}$$

$l(a)$, the survival at age a is:

$$l(a) = \frac{X(a) + Y(a)}{X(0) + Y(0)}$$

Parasite transmission is calculated in terms of the frequency of infected individuals. We calculate the optimal host senescence by maximizing the intrinsic rate of increase of the host. The density-dependent birth rate is only used to calculate the epidemiological equilibrium, allowing the calculation of survival, and is not included directly in the calculation of fitness:

$$r = \int_0^\infty l(a)\ b\ da$$

We calculate the evolution of host senescence across a range of values for virulence, presenescent mortality and s. We do not test all possible values, but aim to demonstrate some of the evolutionary outcomes predicted by the model.

We found that for particularly large values of virulence we were unable to reach the equilibrium for the force of infection after a large number of iterations. These values of virulence were therefore not included in the results.

Generally, increasing virulence resulted in the evolution of higher senescence. Increasing s increases the equilibrium senescence (Fig. 3.1a), which is associated with a decrease in prevalence (Fig, 3.1c). Increasing virulence increases the differences between the optimal senescence for the different values of s.
We see that increasing presenescent mortality increases the senescence (Fig. 3.1b), which is associated with lower prevalence (Fig. 3.1d). Senescence becomes more sensitive to virulence as presenescent mortality increases.

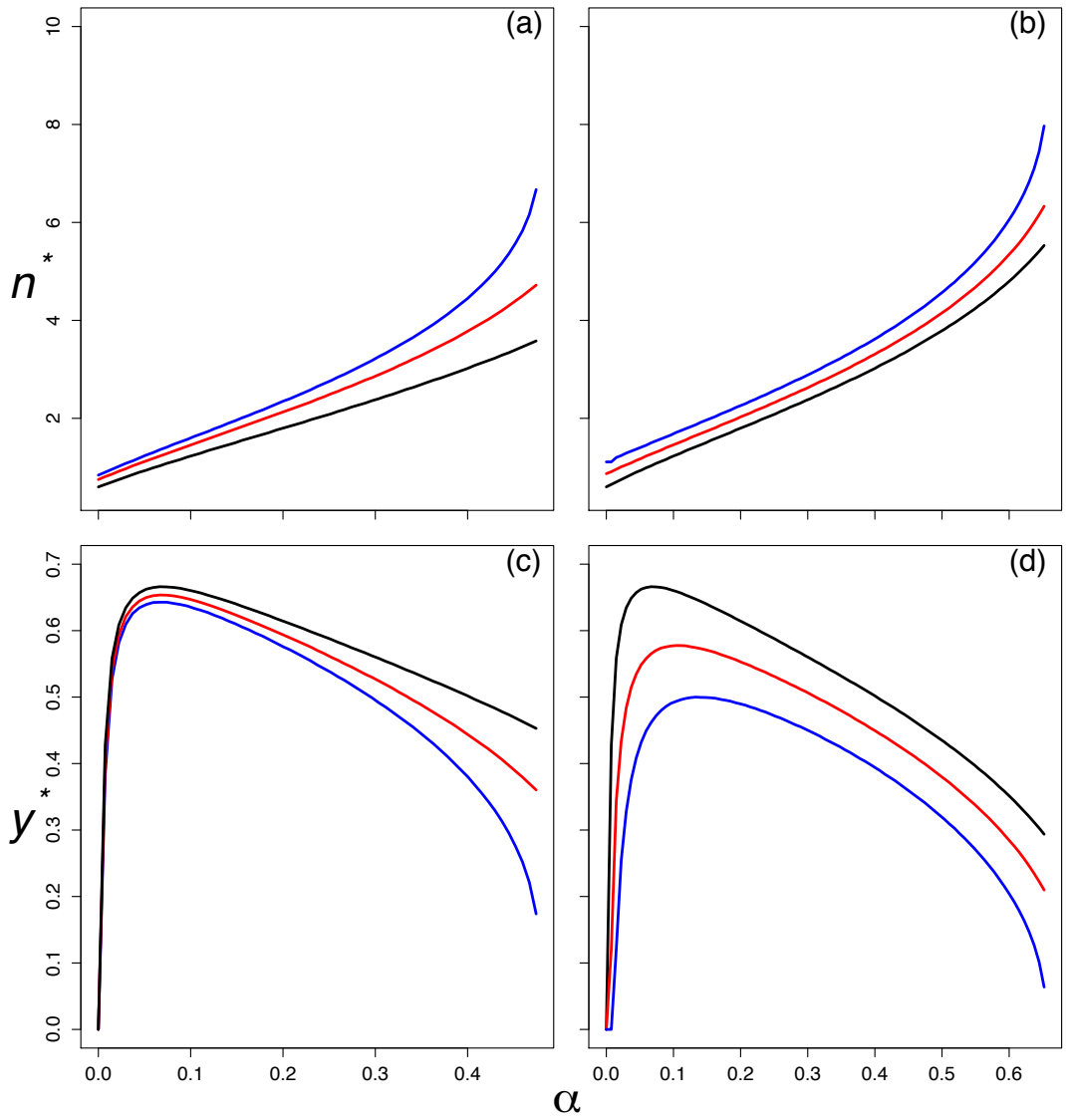

Figure 3.1: Evolved senescence (a and b) and associated levels of prevalence (c and d) for different values for virulence, s (a and c) and presenescent mortality (b and d). μ_0 = 0.07 (black), 0.11(red), 0.15(blue); s = 0.075 (black), 0.1375 (red), 0.2 (blue). κ = 0.001, β_0=1.7, b_0=0.28, η=0.5. For a and c, μ_0 = 0.07, and for b and d, s = 0.075.

3.3 Discussion

Our results are in accordance with the classical predictions of Williams (1957) and Medawar (1952), and suggest that higher extrinsic mortality in the form of parasitism forces the evolution of higher senescence. Therefore, incorporating epidemiological dynamics does not change our intuition about how parasites as a source of extrinsic mortality should influence senescence evolution. Additionally, we see that presenescent mortality and virulence act in similar ways in shaping the evolution of senescence.

A primary limitation of our study is that we are unable to calculate the equilibrium senescence until the threshold for parasite extinction. Whether our predictions apply to relatively higher values of virulence when prevalence is particularly low remains to be tested. In age-independent models increasing a constant mortality term will reduce the maximum virulence for which a parasite can invade a host population (Boots & Sasaki 2003). Thus, the maximum virulence for which the parasite can invade is likely to decrease as presenescent mortality increases. If this were the case, we would see that higher presenescent mortality could in fact select for lower senescence for high values of virulence, since the parasite would not be able to invade the host population. Therefore at particularly higher levels of virulence, the evolved level of senescence would drop to the equilibrium found at $\alpha = 0$.

As tested by Williams & Day (2003), there are likely to be interactions between sources of extrinsic mortality and the internal condition of the individual. This can be of particular importance in host-parasite systems. Experimental evidence suggests that parasites can show plasticity for virulence depending on the age of the host they infect (Restif & Kaltz 2006), whilst other studies show virulence can depend on environmental conditions (Brown et al. 2000; Brown et al. 2003). Although these studies either don't test if increases in virulence are associated with higher transmission, or consider the virulence transmission trade-off in terms of transmission mode (horizontal transmission being more infectious than vertical transmission), they suggest a possible extension of the work here. We

test how senescence evolves for a range of fixed values of virulence, however a more accurate assumption might be that a parasite's virulence depends (via plasticity) on the age at which the host is infected (Kysela & Tuner, *unpublished*). For example, a parasite may invest more in transmission (thereby increasing it's virulence) when it infects old individuals, and less when infecting younger individuals. In contrast to previous studies, we suggest accounting for plasticity for virulence in response to age at infection in the context of a parasite exhibiting horizontal transmission only.

Assumptions about the evolutionary constraints experienced by the host and parasite warrant further consideration. The trade-offs between reproduction and survival differ between the host and the parasite; we assume that the host can reproduce without showing senescence, whilst the parasite must decrease the survival of the host in order to be transmitted. Although for the parameters we use, the host always evolves to increase its mortality rate with age, its worth noting that parasites can evolve to be benign given the possibility of transmission without increasing the host's mortality rate (i.e. by allowing for transmission when $\alpha = 0$) (Koella, *unpublished*). Therefore, it is possible that in the context of this model, the system may be characterized by both no senescence and no virulence depending on the formulation of the trade-off between components of fitness in each species.

In summary, we demonstrate that the classical theory of senescence applies to host-parasite systems, when infection increases host mortality and occurs horizontally. Future work should test the validity of our predictions experimentally. An important challenge in relating the theory to data, would be determining whether a virulence transmission trade-off is present, when using an experimental system where transmission occurs horizontally only.

Chapter 4. Coevolution between parasite virulence and host senescence

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Abstract

Coevolutionary interactions are an important determinant of the evolution of host and parasite life histories, yet there is no theory of coevolution that accounts for age-dependent increases in the mortality rate of the host (senescence). We use an age-structured epidemiological model to calculate the optimal parasite virulence and host senescence whilst accounting for coevolutionary dynamics. The model predicts that allowing for coevolution results in higher equilibrium levels of virulence and senescence, than when evolution occurs in the parasite alone. Furthermore, coevolution may lead to parasite-driven extinction of the host in circumstances that would be defined by an endemic equilibrium in the absence of coevolution. We show that increasing transmission reduces the coevolutionary equilibrium for senescence and virulence, which is due to its role in reducing parasite virulence but having a neutral outcome for senescence evolution.

4.1 Introduction

Since the first application of coevolutionary thinking to theoretical formulations of host-parasite interactions 60 years ago (Mode 1958), the idea that hosts and parasites undergo reciprocal evolutionary change is now widely recognized as an important force in shaping the diversity of tactics employed by hosts and parasites to maximize their fitness. Studies on host-parasite coevolution began with a particular focus on virulence and host ability to reduce parasite burden (May & Anderson 1983). It is now well known that hosts can also mitigate the damaging effects of infection by altering their life history, for example by evolving earlier maturation (Lafferty 1993; Agnew et al. 2000; Hochberg et al. 1992). At the same time, both theoretical and experimental studies suggest that host life history can influence the outcome of parasite evolution (Nidelet et al. 2009; Gandon, Jansen, et al. 2001; Kakehashi & Yoshinaga 1992). This work has since lead to well-developed theory of coevolution between host and parasite life histories (Koella & Restif 2001; Gandon et al. 2002; Restif & Koella 2003b; Ashby & Boots 2015).

Given that evolutionary constraints in the form of genetically determined trade-offs influence the course of evolution (Loeschcke 1987; Roff 1992; Stearns 1992; Lande 1982), they are at the centre of the coevolutionary dynamics of the host and parasite. For the parasite, this constraint comes in the form of the virulence transmission trade-off. This trade-off is based on the assumption that parasite transmission, which depends on within host replication, increases the mortality of the host. Although this relationship has received mixed empirical support, it is generally accepted as the best approximation of the cost of parasite transmission (Alizon et al. 2009). For the host, and what is the central topic of this article, is that an increase in fecundity should be associated with an age-dependent increase in the mortality rate. This age-dependent increase in mortality rate, referred to as senescence, has yet to be accounted for in a coevolutionary theory of hosts and parasites. Williams (Williams 1957) was the first to suggest that increases in fecundity early in life should be associated with an increase in mortality later in life. The assumption of a genetically determined trade-off

between fecundity and survival finds frequent support from experimental data (Rose & Charlesworth 1981; Scheiner et al. 1989; Hughes & Clark 1988). Charlesworth (Charlesworth 1994) provided the first mathematically formalism of senescence evolution, which accounted for population genetics.

The evolutionary theory of senescence suggests that extrinsic mortality factors (for example parasitism), determine where along the trade off between fecundity and survival a phenotype will evolve (Williams 1957). As demonstrated in chapter 3, increasing virulence drives the evolution of higher levels of senescence. Similarly, higher levels of senescence cause the evolution of higher levels of virulence (chapter 2). Parasite evolution is ultimately determined by the average age at infection, since different ages at infection are associated with different background host mortality rates (Chapter 2). Increasing the transmission coefficient independently of virulence (i.e. by manipulating β_0, when the transmission coefficient is given by $\beta = \beta_0\sqrt{\alpha}$) should decrease the age at infection (since it increases transmission) thereby selecting for lower virulence. We would expect that increasing β_0 should increase the equilibrium senescence, since it reduces survival, thereby selecting for increased reproduction early in life. Therefore, manipulating β_0 may have contrasting outcomes for the host and parasite.

Distinguishing between frequency and density-dependent transmission can influence the evolutionary outcome for virulence in age-structured models (Chapter 2). To eliminate any dependency of the optimal virulence on the parameters of host population growth, transmission must be modelled as a frequency-dependent process. An important implication of frequency-dependent transmission is that it enables the parasite to drive the host to extinction (Getz & Pickering 1983; Boots & Sasaki 2003). How the coevolutionary dynamics of senescence and virulence influence the risk of extinction is yet to be tested. Whether we should expect continual increases in each trait until the host is extinct, or coexistence at the equilibrium will depend on the parameters determining host survival and parasite transmission. In standard models, thresholds for parasite-driven extinction depend on the birth and mortality rates

of the host, as well as the parasite's virulence and coefficient of transmission. In the context of the model developed in chapters 2 and 3, we would therefore expect increasing presenescent mortality (host mortality which is independent of parasite infection and senescence) to increase the risk of extinction, since it should not only increase the evolved virulence but also the optimal senescence. Additionally, the balance of how the coefficient of transmission influences virulence and senescence evolution will determine whether an endemic equilibrium is obtainable.

We address the uncertainties outlined above by first calculating how increasing transmission influences host senescence and virulence evolution without the coevolutionary interaction, and then proceed to predict the coevolutionary equilibrium for different values of presenescent mortality and the transmission coefficient.

4.2 The model

We investigate the coevolutionary process by first calculating the optimal parasite virulence given an initial condition for host senescence, and then calculate the optimal host senescence given this level of virulence. This process is repeated until the coevolutionary equilibrium is reached. To check if the host goes extinct, we calculate if the intrinsic rate of increase is < 1 after initial fluctuations, when simulating the epidemiology of the parasite to equilibrium.
We calculate coevolution for various values of β_0 (which controls the average age at infection), and μ_0 (the presenescent mortality rate). Additionally, we verify the role of the transmission coefficient in parasite and host evolution without the coevolutionary interaction.

We calculate parasite evolution as in chapter 2, with the exception that the birth rate is given by the trade-off between host fecundity and senescence as used in

chapter 3. Host evolution is calculated as in chapter 3. Parasite transmission is assumed to be frequency-dependent.

4.3 Results

Without coevolution, increasing β_0 resulted in the evolution of lower virulence (Fig. 4.1), whilst increasing presenescent mortality resulted in the evolution of higher virulence (result not shown). Although we found that increasing β_0 reduced host survival (result not show), it did not alter the optimal senescence (Fig. 4.1).

At the coevolutionary equilibrium, we see that increasing β_0 reduces virulence and senescence (Fig. 4.2). Increasing the presenescent mortality has two outcomes for the coevolutionary equilibrium; it increases both the senescence and virulence, whilst also decreasing the threshold β_0 for parasite driven extinction of the host. We also see that the evolution of the parasite against a static host with the initial conditions (and the highest presenescent mortality rate used to calculate the coevolutionary equilibria) resulted in considerably lower virulence than when the coevolutionary interaction is also accounted for. Additionally, excluding coevolution did not result in host extinction for any value of β_0 (Fig. 4.2). This result suggests that coevolution can drive the host to extinction in situations where an endemic equilibrium is possible without the coevolutionary interaction. Prevalence tends to increase with β_0, and decrease as virulence and senescence increase. There were small, but visible differences, between the equilibrium prevalence for each value of presenescent mortality (Fig. 4.3).

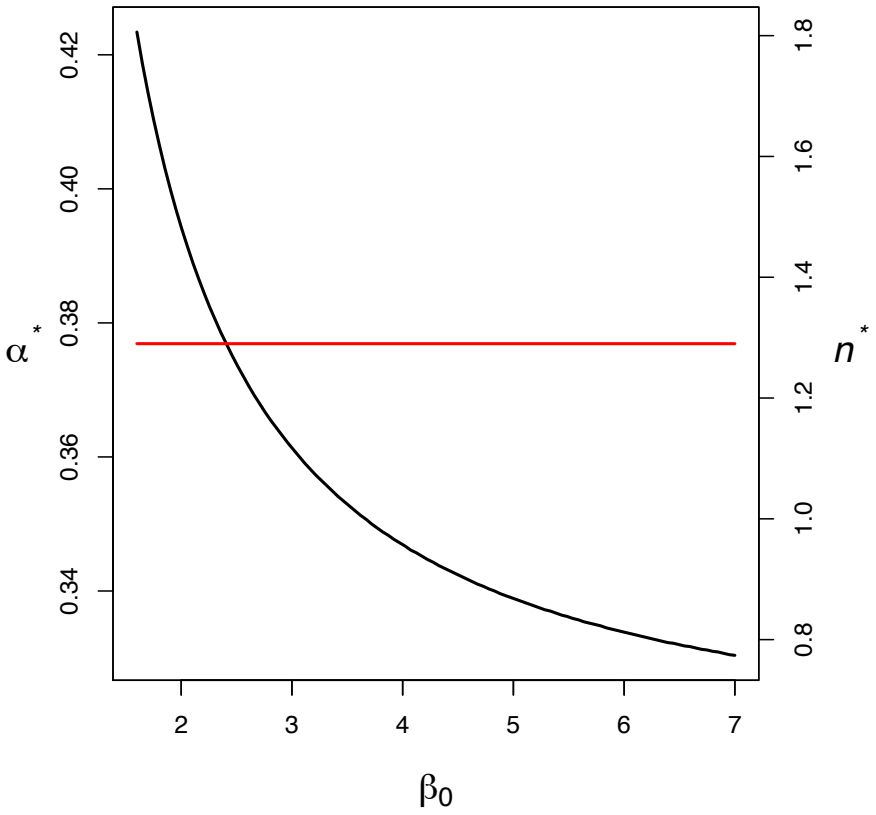

Figure 4.1: Evolutionary equilibria for senescence with a static parasite (red line) and virulence with a static host (black line). κ = 0.0075, b_0 = 0.2, s = 0.35, η = 0.15, μ_0 = 0.07, for the host α = 0.3 and for the parasite n = 3.

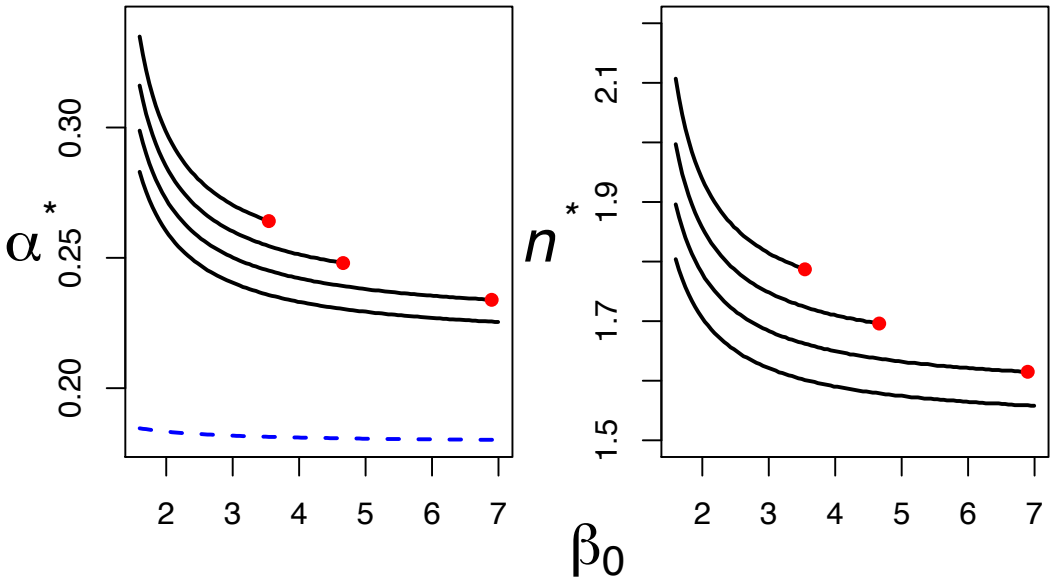

Figure 4.2: Coevolutionary equilibria for virulence (left) and senescence (right) for μ_0 = 0.14 (bottom black line), 0.145, 0.15, 0.155 (top black line). κ = 0.0075, n = 0.7 (initial condition), s = 0.35, η = 0.15, b_0 = 0.01. Virulence evolution against a constant host for the starting conditions for coevolution and μ_0 = 0.155 is shown in blue. The red points show the final endemic equilibria before host extinction.

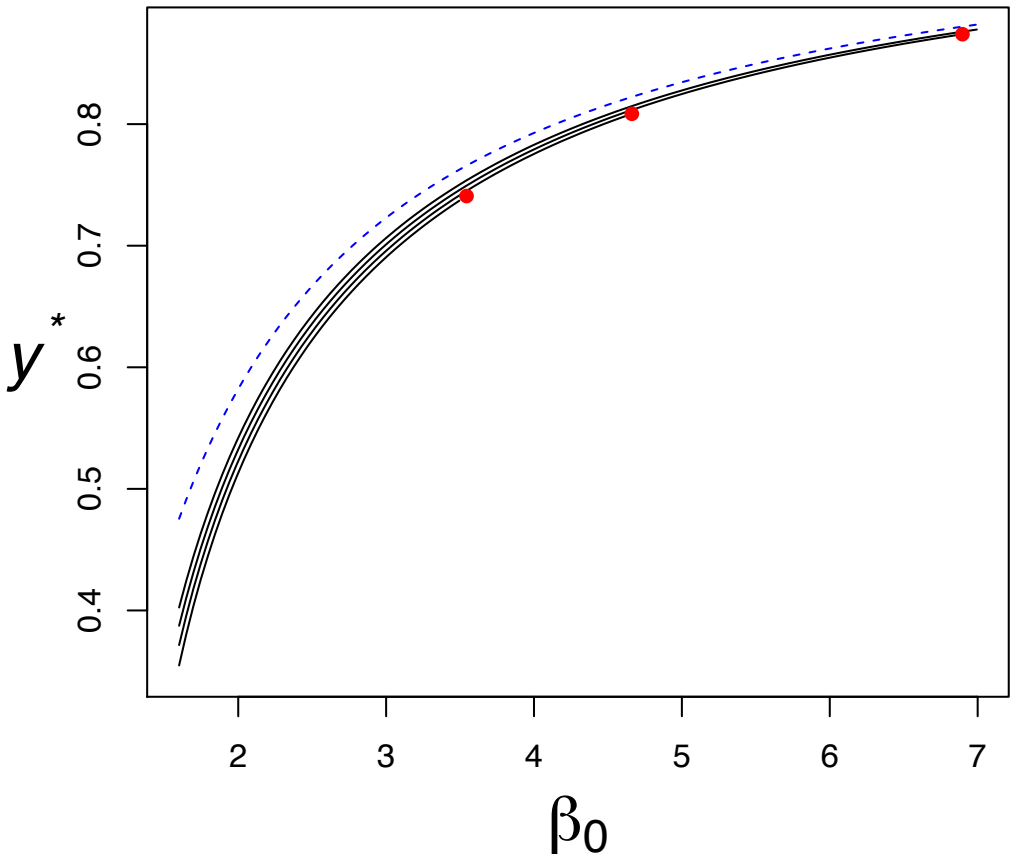

Figure 4.3: The associated parasite prevalence for the equilibria shown in figure 4.2. μ_0 = 0.14 (top black line), 0.145, 0.15, 0.155 (bottom black line). The dashed blue line gives the equilibrium prevalence for the parasite against a static host.

4.4 Discussion

This is the first time a theory of host-parasite coevolution has accounted for continuous age-structure in the host population. This approach allows us to calculate the coevolutionary outcome of host senescence and parasite virulence. We make two interesting predictions: Firstly, accounting for the coevolutionary dynamics can result in extinction of the host population in a system that would otherwise be characterized by an endemic equilibrium. Secondly, we show that transmission influences the evolved virulence, which consequently determines the coevolutionary equilibrium. In models assuming constant host mortality, increasing transmission does not influence virulence evolution, since all ages at infection are associated with the same background mortality rate of the host.

The association of higher levels of senescence with higher virulence and presenescent mortality is consistence with the findings in chapter 3, where the host evolves against a static parasite. Similarly, our findings are in accordance with those in chapter 2, in that increasing senescence increases virulence.
Additionally we retrieve the findings of Boots & Sasaki (Boots & Sasaki 2003); increasing the presenescent mortality rate decreases the threshold coefficient of transmission for host and parasite coexistence, and also forces the evolution of higher virulence, increasing the risk of host extinction.

A surprising prediction of the model is that the transmission coefficient plays no role in the evolution of senescence. Although increasing the transmission coefficient decreases host survival and consequently the intrinsic rate of increase, the highest host fitness is found for the same level of senescence regardless of transmission (when there is no parasite evolution). It follows, that increasing the transmission coefficient reduces survival by the same amount for each value of n. Since the birth rate is constant with age, the intrinsic rate of increase exhibits the same behaviour. This suggests that extending the model to allow for age-dependent fecundity may alter our predictions. As we show in chapter 3, virulence does influence senescence, implying that increasing virulence reduces survival differently for each value of n. Perhaps a more

pertinent question is then: why do we observe these different patterns for virulence and the coefficient of transmission? The main difference between increasing virulence and the transmission coefficient is that, for the former, prevalence decreases, whilst for the latter, it increases. The question can then be rephrased in terms of prevalence: Why does decreasing prevalence (but with increases in virulence) increase senescence, but increasing prevalence for a constant virulence does not? At this point, the answer to that question is not clear.

In the only review on the role of parasites in the extinction of their hosts, coevolutionary dynamics were absent (De Castro & Bolker 2005). Indeed, we are aware of only one other study, which demonstrates the role of coevolution in host extinction (Ashby & Boots 2015). Our results are dependent on the assumption of frequency-dependent transmission; for density-dependent transmission, the parasite will always go extinct before the host (Anderson & May 1979; May & Anderson 1979; Anderson & May 1978; May & Anderson 1978). Density-dependent transmission may also complicate the outcome of coevolution. As shown in chapter 2, host fecundity influences parasite evolution since it changes the average age at infection. When a parasite evolves increased virulence, this should select for an increase in senescence. This higher level of senescence would cause an increase in fecundity (due to the trade-off), lowering the average age at infection. Given a strong enough trade-off between fecundity and senescence, there is the potential to select for decreased virulence in the parasite. This decrease in virulence would then select for lower senescence, which would reduce the birth rate, decreasing the age at infection, thereby selecting for higher virulence. Consequently, density-dependent transmission could result in coevolutionary cycling of virulence and senescence.

In summary, our results suggest that accounting for the coevolutionary dynamics of virulence and senescence provide variations of the predictions of evolutionary models of either the host or parasite only. This work would benefit from a closer investigation of why parasite virulence, but not transmission, influences the evolution of senescence. Additionally, implementing the framework presented

here in the context of density-dependent transmission may reveal less trivial coevolutionary dynamics, than when transmission is frequency-dependent.

Chapter 5. The impact of food availability and sex on resistance and tolerance of yellow fever mosquitoes against microsporidian infection

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Abstract

Although tolerance and resistance are becoming increasingly recognised as distinct defence strategies against parasite infection, evidence for the factors that shape tolerance and its costs is scarce in animal systems. We measured the resistance (the inverse of parasite burden) of the yellow fever mosquito to a microsporidian parasite, and its tolerance (Least squares mean) with regard to longevity and with regard to fecundity. Although neither sex nor larval food influenced the two measures of tolerance or resistance, the reproductive behaviour of female mosquitoes (blood feeding and egg laying) influenced longevity tolerance and resistance. Individuals that laid eggs had higher tolerance than those that did not blood feed for both larval food treatments. We also found a cost of tolerance, higher longevity tolerance was associated with a shorter lifespan in the absence of parasites, and this trade-off was influenced by an interaction between food availability and the sex of the host. We found no genetic correlation between either measure of tolerance and resistance, and neither covariance was influenced by food or sex. This result suggests that resistance and tolerance can evolve independently. A cost of tolerance in terms of fitness in the absence of infection may maintain variation for tolerance when the host's environment has fluctuated between being infested and parasite-free over evolutionary time. Our results therefore provide a possible explanation for why we frequently see a genetic basis for tolerance in animal systems, even though theoretical studies predict that it should become fixed.

5.1 Introduction

Hosts can diminish the negative impact of parasite infection by reducing the parasite burden (that is, by being resistant), or by reducing the impact of a given parasite burden (that is, by being tolerant). While plant biologists first appreciated the importance of tolerance (Caldwell et al. 1958; Schafer 1971), tolerance of animals has been receiving increasing interest (Råberg et al. 2007; Råberg et al. 2009; Read et al. 2008). For evolutionary biologists, distinguishing between the two defence strategies is crucial, for they lead to qualitatively different evolutionary outcomes of the host-parasite interaction (Roy & Kirchner 2000; Restif & Koella 2003a; Best et al. 2008). On the one hand, resistance decreases the parasite's fitness, so it is expected to lead to continued coevolutionary cycles between the host and parasite (Roy & Kirchner 2000). Indeed, we often observe genetic variation for resistance in host populations (Webster & Woolhouse 1999; Lambrechts et al. 2006). On the other hand, tolerance need not affect the parasite's fitness. Although it can select for increased parasite growth in some situations, tolerance is generally predicted to go to fixation in the host population (Roy & Kirchner 2000; Miller et al. 2005). Nevertheless, there is often, but not always (Lefèvre et al. 2010; Hayward et al. 2014), genetic variation for tolerance in animal systems, in laboratory (Råberg et al. 2007; Vale & Little 2012; Parker et al. 2014) and natural populations (Blanchet et al. 2010; Mazé-Guilmo et al. 2014), as well as in clinically relevant diseases (Regoes et al. 2014).

In addition to the genetic variation of tolerance and resistance, the two traits differ among environmental conditions. Resistance depends on food availability in mosquitoes infected with malaria parasites (Lambrechts et al. 2006) and in *Drosophila melanogaster* infected with *Providencia rettgeri* (Howick & Lazzaro 2014). Though there are only a few studies available, these suggest that tolerance can change in response to food availability (Howick & Lazzaro 2014), food type (Sternberg et al. 2012) and host sex (Vincent & Sharp 2014). In particular, resources may influence resistance and tolerance differently, so that relative allocation of the host's resources to one or the other defence mechanism

should depend on the environment. Details of the allocation to resistance and tolerance, like the allocation to other life-history traits, will depend on how they are linked by internal constraints. However, there is no consensus on how tolerance and resistance are correlated. Whilst the correlation between tolerance and resistance was negative in two studies (Råberg et al. 2007; Vincent & Sharp 2014) it was positive (Howick & Lazzaro 2014) or insignificant (Mazé-Guilmo et al. 2014; Lefèvre et al. 2010) in others.

Links of resistance and tolerance to other traits, that is the costs of defence, will also have a large impact on evolution. While there are numerous studies documenting that resistant genotypes have lower fecundity when uninfected than sensitive ones (Cotter et al. 2004; Webster & Woolhouse 1999; Groeters et al. 1994), analogous data for tolerance, showing for example, that increased longevity of infected individuals might lead to a shorter lifespan of uninfected ones, are largely missing in the animal literature (although one study found no correlation between the fecundities of infected and uninfected individuals (Howick & Lazzaro 2014).

Furthermore, the ideal definition of fitness is likely to depend on the context (Raberg *et al.,* 2009). In clinically relevant parasites, for example, host lifespan is likely to be more important than number of offspring, whilst in the context of agriculture, yield is of most interest. We should therefore have measures of tolerance for both traits. Whether these measures of tolerance are correlated will have relevance in an evolutionary context, since in population genetic models, fitness is determined by survival and fecundity. However, no studies have measured the correlation between the tolerances with regard to longevity and fecundity.

Finally, the environment may not only influence the two defence strategies, but may also determine their evolutionary costs, just as correlations between other life history traits can change depending on environmental conditions, which in turn can influence the outcome of long-term evolution (Sgrò & Hoffmann 2004). However, in the context of tolerance and resistance, there is little evidence on

how the environment influences the correlation between the two traits, or between tolerance and other host life history traits. In the only study on the influence of food availability on the costs of tolerance, a change in diet had no influence on the correlation between resistance and tolerance (Howick & Lazzaro 2014). The only study investigating the role of host sex in the costs of tolerance showed no differences between sexes, with negative correlations between tolerance and resistance for males and females (Vincent & Sharp 2014).

We address four main questions about resistance and tolerance (in terms of fecundity and longevity) of the mosquito *Aedes aegypti* infected with the microsporidian *Vavria culicis*: (i) Does food availability (larval food and adult blood feeding) or host sex influence tolerance and resistance? (ii) Is longevity tolerance influenced by reproduction? (iii) Are the two measures of tolerance (tolerance measured in terms of longevity or in terms of fecundity) correlated and are they costly in terms of resistance and other host life history traits? (iv) Are these correlations influenced by food availability and host sex?

5.2 Materials and methods

Organisms

We used the UGAL strain of *Aedes aegypti* (from P. Guerin, University of Neuchâtel) and its microsporidian *Vavraia culicis* (from J. Becnel, USDA Gainesville). *Vavraia culicis* is an obligate unicellular parasite, which is transmitted from an infected larva to other larvae when it dies or from an infected mother to her offspring if she deposits spores on her eggs. *Aedes aegypti* occurs throughout the tropics and sub-tropics and is an important vector of clinically relevant diseases such as yellow fever and dengue.

Experimental design

The experiment was run in a climate-controlled chamber at 26°C, 70% humidity with a 12h:12h light-dark cycle.

To investigate the genetic basis of tolerance and resistance, we used full-sib families. To generate the maternal generation, we reared larvae separately in 12 well plates, with each well containing 3ml of deionized water, on the standard food levels used in our lab (Tetramin fish food; day 1: 0.06mg, day 2: 0.08mg, day 3: 0.16mg, day4: 0.32mg, day 5: 0.64mg, day 6 or later: 0.32mg). Pupae were allowed to emerge in separate 300ml cups. Cups were sealed with netting and cotton wool soaked in 10% sugar solution was placed on the top of each cup. Each female was then used to create a family. Two days after emergence, two males were selected haphazardly from the mosquito colony and added to each cup containing a female. Females were transferred to egg-laying cups containing damp filter paper and offered a blood meal. After eggs were produced, the filter paper on which the eggs were laid was stored at 27 degrees and each female was transferred to a new cup and offered another blood meal. This was repeated until all the females were dead (note, that *Aedes aegypti* require a blood meal before each clutch, but only need to mate once to reproduce for life).

For the offspring generation, food and parasite treatments were administered to each family in a factorial design. Individuals were reared individually in 12-well plates on either high (the standard amount of food) or low food (half the standard); and infected or not with *Vavraia culicis*. For the infection, the larvae were exposed 2 days after hatching to 20000 spores. Individuals were allowed to emerge in separate cups and were then fed with 10% sugar solution. Survival was checked every 24 hours. Two males from the colony were added to each cup containing a female, and a blood meal was offered two weeks after emergence. We recorded whether females successfully fed or not.

Statistical analysis

For our analysis, we defined an individual's resistance as the inverse of the number of spores found at the time of death. Note that in this and other studies, the average number of spores remains almost constant once adults are older than about two weeks. Since almost all individuals (> 98%) lived longer than two weeks, resistance and age were not related.

We measured tolerance in terms of adult longevity and fecundity. Tolerance is not a measure of individuals, but of genetically related groups. It can be measured as the slope of the regression of longevity or fecundity on the number of spores (Raberg et al. 2009) or as the least-square mean after controlling for number of spores (and other factors) (Howick & Lazzaro 2014). We chose the latter approach for our analysis. An important difference is that the LS means take account not only of the slope, but also the intercept of the regression relating longevity (or fecundity) to parasite burden.

Measures of resistance and tolerance

To test for a genetic basis for tolerance and resistance, and whether these traits were influenced by food availability and host sex, we constructed two linear mixed effects models in R, using the nlme package. Spore data were log-transformed. The residuals and the distribution of random effects were checked for the model's assumptions. Family, and the interaction between family and parasite burden were modelled as random effects. We constructed two further models using only the data of females to test if blood-feeding or egg laying influenced longevity tolerance or resistance. In these models, we included a variable 'reproductive behaviour' with three levels: no blood-feeding, blood-feeding without egg-laying, and blood-feeding with egg-laying. To test for specific differences in tolerance between the different types of reproductive behaviour, we used the LS means function in R to conduct a Tukey's test. In the analyses that included both males and females, we used all female individuals (i.e. those which accepted a blood meal and those that did not), since all the

individuals experienced the same environment (they were all offered a blood meal).

To calculate the tolerance for each family to investigate its costs, we used the least squares means (LS means) for longevity and fecundity when infected. For longevity, these were obtained from a linear model containing food, sex, parasite burden and family, as well as the interactions between all factors. A similar model without males and sex as a factor was used to calculate fecundity tolerance; in this model we only included individuals that accepted a blood meal. We obtained an estimate for each family, at each level of food and sex. Since the calculation of these LS means relies on an adequate range for parasite burden within a family, families that had a range less than 20% of the median range across all families and treatments were removed from the analysis. For the final analyses of longevity tolerance and resistance 12 families were used. For fecundity tolerance, this was reduced to 10 families, for in one family the blood-fed females had a low range for parasite burden and in one family the standard error of the LS mean implied negative fecundity.

For the definitions and analyses of resistance and tolerance, we assumed that the parasite load was independent of the mosquito's age. Since all mosquitoes had been exposed to spores at least 20 days before dying, and since the parasite loads becomes independent of age about 14 days after exposure (Zeller and Koella, in press), this assumption does not affect our conclusions.

Costs of resistance and tolerance

We conducted five ANCOVAs. Two ANCOVAs were used to assess how food availability and host sex influenced the correlation between longevity tolerance and the mean resistance per family, and between longevity tolerance and the mean longevity of unexposed individuals per family. A further three ANCOVAs were used to assess the role of food in the correlation between fecundity tolerance and longevity tolerance, fecundity tolerance and resistance, and finally

between fecundity tolerance and the mean fecundity of unexposed individuals per family.

5.3 Results

Genetic and environmental effects

Resistance ranged from families with 0.621 parasites to families with 4.777 parasites (log-transformed) and longevity tolerance ranged from families with a LS mean longevity of 24.923 to 52.814 days. Fecundity tolerance ranged from families with a LS mean fecundity of 16.108 to 54.934 eggs per females. The differences among families were statistically significant for resistance ($p = 0.002$, table 5.1) and longevity tolerance ($p < 0.001$, table 5.2), suggesting a genetic basis for these traits. However, the large variability of fecundity within families did not allow us to conclude a genetic basis for fecundity tolerance ($p = 0.931$, table 5.4).

Neither food nor sex had an influence on longevity tolerance, fecundity tolerance or resistance. The reproductive behaviour (blood feeding and egg laying) of female mosquitoes influenced longevity tolerance (Fig. 5.1, Table 5.3) and had a combined effect with larval food availability on resistance (Fig. 5.1, Table 5.5). Generally the highest LS mean longevity was found in individuals that laid eggs (see the appendix for the post hoc analysis for longevity tolerance).

	Parasite burden (n=354)	
Factor	**χ2**	**p**
Family	9.25	**0.002**
Food	1.35	0.244
Sex	0.34	0.558
Food*Sex	1.78	0.182
Family * Food	5.64	0.060
Family * Sex	10.85	**0.004**

Table 5.1: Statistical summary for resistance. Family is a random effect.

	Longevity (n=354)	
Factor	**χ2**	**p**
Family	14.64	**<0.001**
Food	0.90	0.344
Sex	0.76	0.384
Parasite Burden	<0.01	0.985
Parasite Burden * Family	12.65	**0.002**
Parasite Burden*Sex	0.83	0.362
Parasite Burden * Food	2.74	0.098
Sex * Food	1.17	0.278

Table 5.2: Statistical summary for longevity tolerance. The significant interaction between family and parasite burden indicates a genetic basis for longevity tolerance. The three-way interaction between sex, food and parasite burden was not significant and therefore removed from the model. Family and its interaction with parasite burden are random effects.

	Longevity (n=161)	
Factor	**χ^2**	**p**
Family	0.55	0.457
Food	0.17	0.678
Reproductive behaviour	0.73	0.695
Parasite burden	13.77	**<0.001**
Family * parasite burden	5.35	0.069
Reproductive behaviour * parasite burden	24.09	**<0.001**
Food * parasite burden	2.42	0.120
Reproductive behaviour * food	3.19	0.203

Table 5.3: Statistical summary of the role of female reproductive behaviour (blood feeding and egg laying) in longevity tolerance. The interaction between reproductive behaviour and parasite burden suggests whether a blood meal was accepted or not and if this resulted in egg laying, influenced longevity tolerance. The three-way interaction between reproductive behaviour, food and parasite burden was not significant and therefore removed. Family and its interaction with parasite burden are random effects.

	Fecundity (n=161)	
Factor	**χ^2**	**p**
Family	0.01	0.931
Food	13.18	**<0.001**
Parasite burden	1.49	0.222
Parasite burden * family	3.33	0.189
Parasite burden * food	2.32	0.128

Table 5.4: Statistical summary for fecundity tolerance. Family and its interaction with parasite burden are random effects.

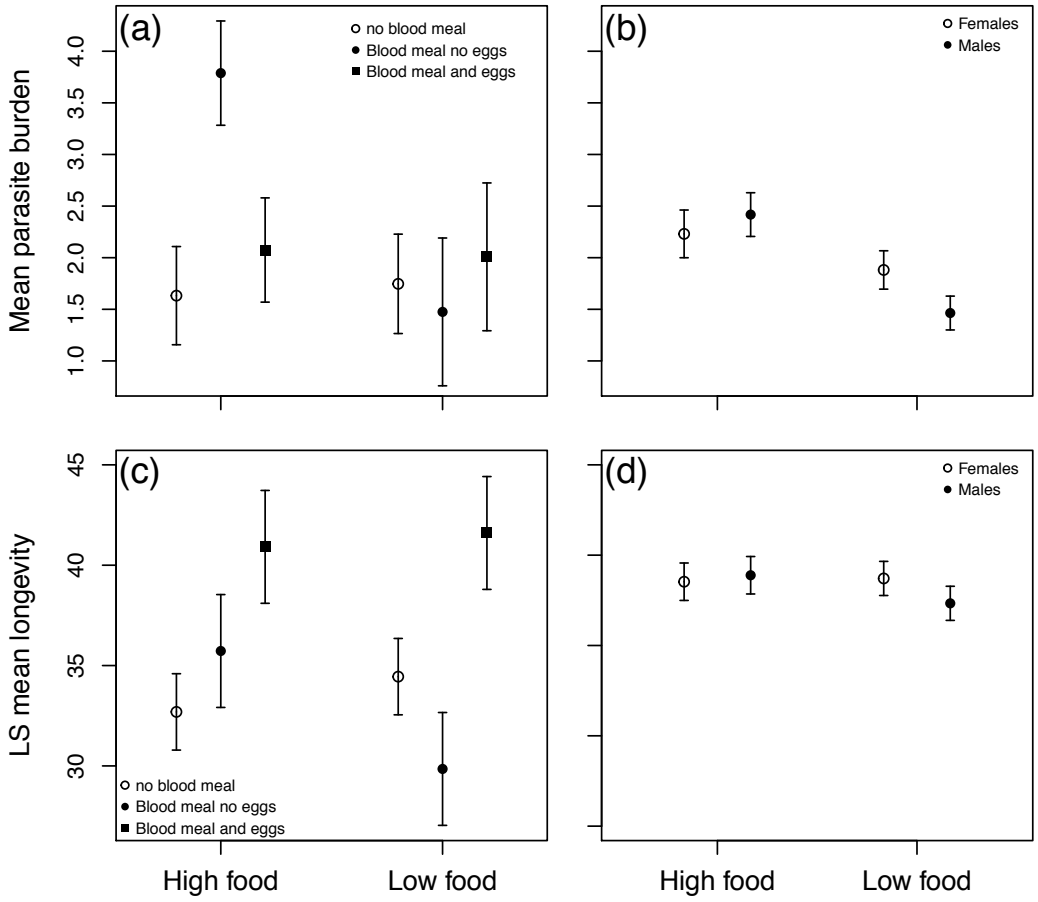

Figure 5.1: a) Log parasite burden for female mosquitoes for high and low food and reproductive behaviour. b) Log parasite burden for males and females reared on high and low food. c) LS mean longevity for the different reproductive behaviours for female mosquitoes for high and low food. The LS means were calculated from a linear model containing food, reproductive behaviour (no blood feed, blood feed without eggs, blood feed with eggs) and parasite burden, as well as the interaction between the three factors. d) LS mean longevity for male and female mosquitoes for high and low food. LS means were calculated from a linear model containing food, sex and parasite burden, as well as the interaction between the three factors.

	Parasite burden (n=161)	
Factor	**χ^2**	**p**
Family	3.77	0.052
Food	<0.01	0.999
Reproductive behaviour	10.08	**0.006**
Food * reproductive behaviour	6.31	**0.043**

Table 5.5: Statistical summary of the role of reproductive behaviour in resistance. Family is a random effect.

Correlations and costs

We found a cost of longevity tolerance in terms of longevity in the absence of infection for high food females only (r = -0.672) (Fig. 5.2), and we found a significant interaction between longevity in the absence of infection, sex and food, suggesting that food and sex had a combined influence on this cost of tolerance (Table 5.6). Neither food nor sex influenced the covariance between longevity tolerance and resistance, and we found no correlation for any single treatment (Table 5.7, Fig. 5.3). The ANCOVA suggests a positive relationship between fecundity tolerance and longevity tolerance (Table 5.8, Fig. 5.4). Excluding the family with an unusually large variance resulted in a significant positive correlation between the two measures of tolerance for the high food treatment (r = 0.783 with p = 0.0216). We found no correlation between fecundity tolerance and either parasite burden or fecundity in the absence of infection; moreover food played no role in the covariance between any of these traits (tables 5.9 and 5.10, Fig. 5.4).

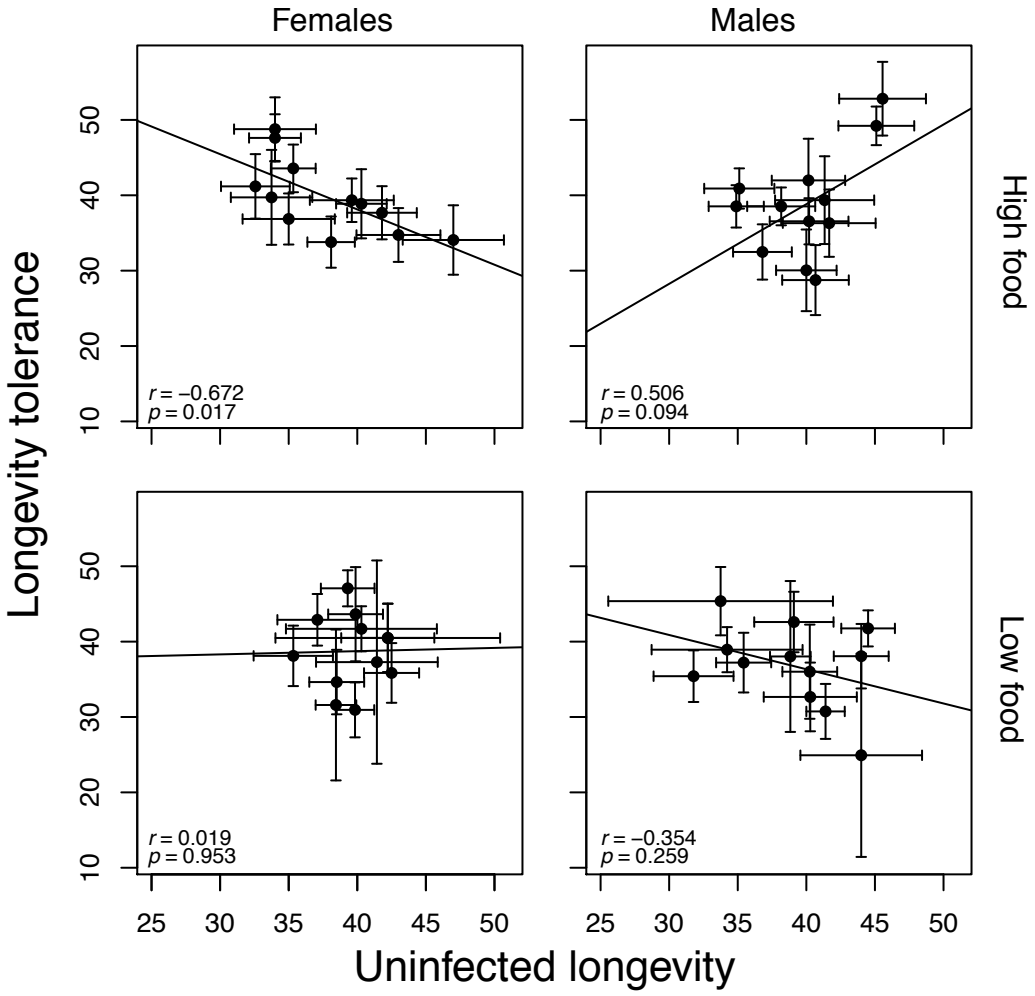

Figure 5.2: Correlations between longevity tolerance and the mean longevity in the absence of parasite infection for males and females, for high and low food.

	Longevity Tolerance		
Factor	**F**	**SS**	**P**
Uninfected longevity	<0.01	0.21	0.931
Sex	1.81	50.29	0.187
Food	0.48	13.36	0.493
Sex*Food	4.95	137.83	**0.032**
Uninfected longevity*Sex	1.66	46.17	0.205
Uninfected longevity*Food	0.54	14.92	0.469
Uninfected longevity*Food*Sex	5.19	144.59	**0.028**
Error		1114.26	

Table 5.6: Statistical summary for the analysis of covariance for longevity tolerance and longevity in the absence of parasite infection.

	Longevity Tolerance		
Factor	**F**	**SS**	**P**
Parasite burden	2.23	72.1	0.143
Sex	0.38	12.4	0.539
Food	1.48	47.7	0.232
Sex*Food	1.36	43.8	0.251
Parasite burden*Sex	0.34	10.9	0.565
Parasite burden*Food	1.42	46	0.240
Parasite burden*Food*Sex	1.61	52.1	0.212
Error		1294	

Table 5.7: Statistical summary for the analysis of covariance for longevity tolerance and resistance.

	Fecundity tolerance		
Factor	F	SS	P
Longevity tolerance	4.91	208.25	**0.044**
Food	0.04	1.54	0.851
Longevity tolerance * Food	0.28	11.90	0.605
Error		594.13	

Table 5.8: Statistical summary for the analysis of covariance for fecundity tolerance and longevity tolerance.

	Fecundity tolerance		
Factor	F	SS	P
Parasite burden	0.01	0.72	0.919
Food	1.86	126.41	0.194
Parasite burden * Food	0.14	9.21	0.718
Error		952.71	

Table 5.9: Statistical summary for the analysis of covariance for fecundity tolerance and parasite burden.

	Fecundity tolerance		
Factor	F	SS	P
Uninfected fecundity	0.12	8.43	0.731
Food	0.07	4.56	0.800
Uninfected fecundity * Food	0.07	4.86	0.794
Error		956.83	

Table 5.10: Statistical summary for the analysis of covariance for fecundity tolerance and fecundity in the absence of parasite infection.

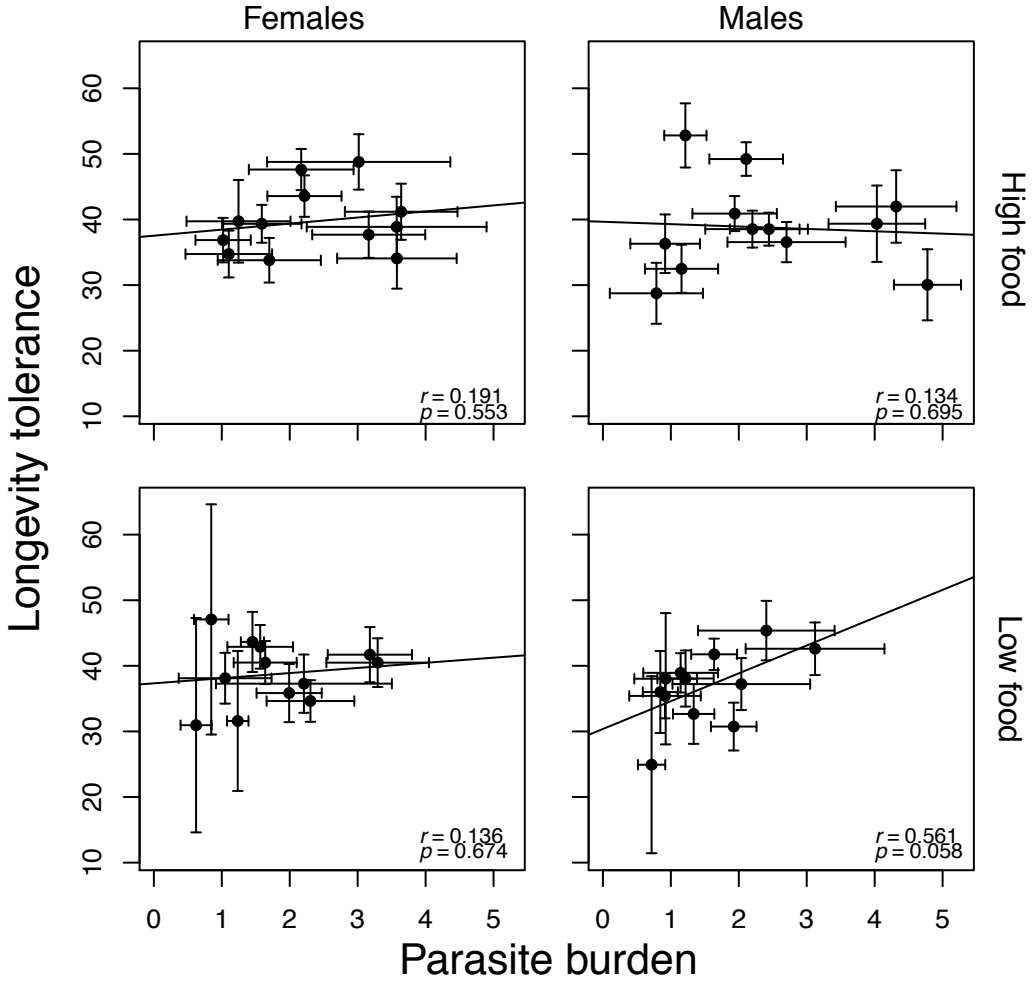

Figure 5.3: Correlations between resistance (parasite burden) and longevity tolerance for males and females, for high and low food.

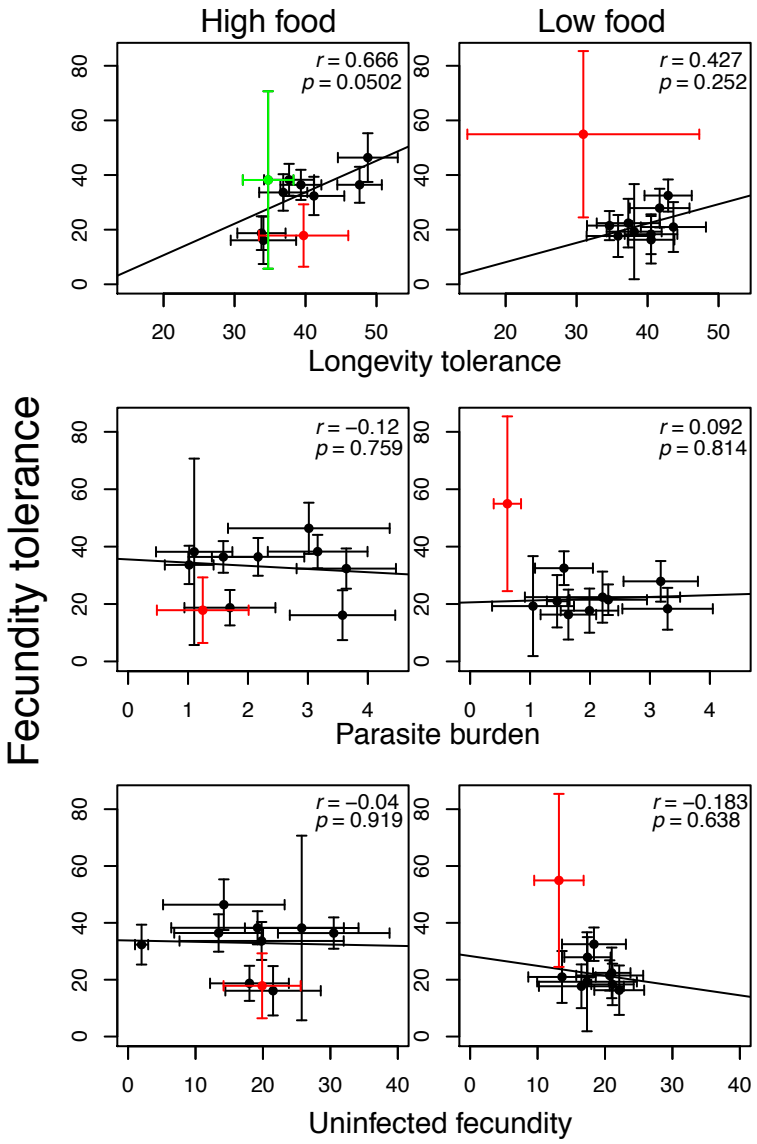

Figure 5.4: Correlations between uninfected fecundity, resistance (parasite burden) and longevity tolerance with fecundity tolerance for females, for high and low food. The family plotted in red was not included when calculating the ANCOVAs and regressions since it has such a large influence on the correlations for low food. It is worth noting that for fecundity tolerance and longevity tolerance for high food, removing the family with the large variance (plotted in green) resulted in $r = 0.783$ with $p = 0.0216$.

5.4 Discussion

Our results show that the adult feeding and reproductive behaviour of female *Aedes aegypti* mosquitoes influences their longevity tolerance and resistance to parasite infection. In contrast, juvenile food availability did not influence longevity tolerance (in males or females) or fecundity tolerance. Our results also suggest that host sex has no influence on longevity tolerance, in contrast to a similar study, which used *Drosophila* as an experimental system (Vincent & Sharp 2014).

Laying eggs was associated with higher longevity tolerance, but did not increase resistance. This may have consequences for transmission, since reproducing individuals were able to tolerate infection (in terms of longevity) more than ones that did not reproduce. Only reproducing individuals transmit the parasite before death (for they deposit the spores on the eggs). Since individuals that laid eggs also live longer than those not reproducing, this allows further opportunities for egg laying, and subsequently further transmission. This is to say, reproducing individuals not only laid more eggs simply because they can deposit spores before death, but also because they tolerate infection better than those which do not reproduce.

We found a positive relationship between fecundity tolerance and longevity tolerance, which to our knowledge has not been shown before using an animal system. Although we did not find a significant family effect for fecundity tolerance, this does not necessarily imply there can be no genetic correlation with other traits, since in quantitative genetics, a genetic correlation only relies on the variance components for the two traits being greater than zero. Thus, rather than a possible trade-off between the two measures of tolerance, we found that the two measures act together to increase the reproductive success of infected individuals. This may not only have important implications for the evolution of tolerance, but also for the ability of female mosquitoes to release spores into the environment before death.

We found no correlation between either measure of tolerance and resistance, which reflects the findings of at least two previous studies (Mazé-Guilmo et al. 2014; Lefèvre et al. 2010), but is in contrast to several others (Råberg et al. 2007; Vincent & Sharp 2014; Howick & Lazzaro 2014). Given that one defence strategy makes the other redundant and therefore not worth the cost of maintaining, we might expect a negative correlation between resistance and tolerance. However, theoretical studies predict that we shouldn't necessarily find a correlation between the two defence traits (Restif & Koella 2003b; Mauricio et al. 1997). Differences in how tolerance is measured across studies may also provide an explanation for this discrepancy. For example 'point tolerance' estimates (fitness at a given parasite burden) will give different results to 'range tolerance' (the slope relating parasite burden to host fitness for a genotype), when the intercepts are not same for all genotypes in each study (Little et al. 2010). Furthermore, details of the host parasite relationship can change the investment in each defence strategy. For example selection for resistance to one parasite can alter resistance and tolerance to others (Ayres & Schneider 2008). However, we did find that longevity tolerance was costly in terms of longevity in the absence of parasites. This trade off may provide an explanation as to why we often find a genetic basis for tolerance in animal systems. If the host's environment has always been infested with parasites, we would expect that the most tolerant individuals produce the most offspring; in this case we would expect tolerance to become fixed. However, if the presence of parasites in the host environment has fluctuated over time, there will have been periods in which individuals have been selected based on their fitness in the absence of parasite infection. In the context of a trade-off between tolerance and longevity in the absence of infection, different families or genotypes would have had higher fitness depending on whether the environment was infested or not. This moving target of selection would therefore create variation for tolerance, since there is selection for individuals with low tolerance in the absence of parasites, and selection for high tolerance when the environment is infested. This trade-off therefore provides an explanation for why empirical data suggests there is variation for tolerance whilst theoretical work does not. With this is mind, accounting for environmental variation and trade-offs between host defence and host life

history traits should be the next development in the theory of tolerance and resistance evolution.

5.5 Appendix

		High food			Low food	
		no blood meal	blood meal no eggs	blood meal with eggs	no blood meal	blood meal no eggs
High food	no blood meal					
	blood meal no eggs	t = -0.989 p = 0.9208				
	blood meal with eggs	t =-3.505 **p = 0.008**	t = -1.659 p = 0.5613			
Low food	no blood meal	t =-0.631 p = 0.9885	t = 0.404 p = 0.9986	t = 2.487 p = 0.1352		
	blood meal no eggs	t =0.843 p = 0.9588	t = 1.575 p = 0.6166	t = 3.720 **p = 0.0039**	t = 1.301 p = 0.7841	
	blood meal with eggs	t =-3.994 **p = 0.0014**	t = -2.046 p = 0.3223	t = -0.743 p = 0.9761	t = -2.951 **p = 0.0423**	t = -4.104 **p = 0.001**

Table 5.11: Statistical summary for the post hoc analysis of the role of reproductive behaviour in longevity tolerance.

Chapter 6. Summary and future directions

6.1 Summary of results

This thesis has two main implications for our understanding of host-parasite interactions. Firstly, we show that the inclusion of host senescence in epidemiological models changes our intuition regarding parasite evolution. This also has implications for host-parasite coevolution, and has the potential to drive the host population to extinction. Secondly, we demonstrate experimentally that there is a cost of host tolerance in terms of fitness in the absence of infection, and that it is influenced by the environment and host sex.

In classical models of parasite evolution, which do not take account of host age-structure, the transmission function and host population dynamics play no role parasite evolution, as long as the parasite can invade the host population. We show that in age-structured models these aspects of host-parasite interactions can have important implications for parasite evolution. We show that under density dependent transmission, the birth rate and carrying capacity of the host can determine the equilibrium virulence, due to their influence over the average at infection. Under frequency dependent transmission the population dynamics of the host have no influence on parasite evolution. These results suggest that in many systems, careful choice of the transmission function is warranted.

In the third chapter, we extend the model in the second chapter to include host evolution. We find that a central prediction of the evolutionary theory of senescence, that increasing extrinsic mortality increases the evolved senescence, is retrieved in the context of host-parasite interactions. The model predicts that higher levels of virulence force the evolution of higher senescence, and this is

consistent across various levels of presenescent mortality and variations in the trade-off between fecundity and senescence.

In the fourth chapter, we combine the approaches in chapters 2 and 3 to calculate the coevolutionary equilibrium for the host and parasite. The model predicts that coevolution can result in parasite driven extinction of the host, for parameters that result in an endemic equilibrium when coevolution is not accounted for. We show that the coefficient of transmission not only increases the likelihood of extinction but also reduces the parasite's virulence before this point, since it alters the average age at infection.

In the fifth chapter, we continue with the theme of evolutionary costs, but focus on host defence to parasite infection using an experimental approach. We show that tolerance is costly in terms of longevity in the absence of parasite infection, and that this cost depends on larval food availability and host sex. Our results also suggest that the reproductive behaviour of female mosquitoes influences tolerance and resistance. The presence of a genetic constraint between tolerance and fitness in the absence of infection may explain why we detected a genetic basis tolerance, since it would maintain variation for tolerance when the host's environment has fluctuated between being infested and free of parasites.

6.2 Future directions

Although it is well known that higher extrinsic mortality forces the evolution of higher senescence (Medawar 1952, Williams 1957, Charlesworth 1993), this thesis is the first theoretical demonstration of this in the context of host-parasite interactions. More specifically we show that increases in parasite virulence select for higher host senescence. Although there is evidence that reducing host lifespan can result in the evolution of higher virulence (Nidelet et al. 2009), there is no experimental evidence for the influence of parasite virulence on the evolution of senescence. As in Nidelet *et al.*, this could be tested using the protozoan *Paramecium caudatum* and its bacterial parasite *Holospora undulate*. The experiment would involve infecting treatment groups with parasite strains

differing in virulence (defined as the difference in host population density in infected and uninfected groups), and subsequently performing survival analyses for the host.

There is also the possibility to develop the theory in this thesis to incorporate age-dependent rates in host defence strategies. There is wide spread empirical evidence that host resistance can either decrease or increase as a function of age (Hayward et al. 2009; Crailsheim & Riessberger-Gallé 2001). Theoretical evidence suggests that higher levels of quantitative resistance (the ability to control parasite burden) should select for increased virulence (Gandon & Michalakis 2000). Consider an age-related decrease in resistance. When individuals are infected at a young age, they will experience a low background mortality rate, which selects for low virulence. At the same time these individuals will show high levels of resistance, which selects for high virulence. It is not necessarily clear whether the initial condition for the average age at infection will determine the evolutionary equilibrium for virulence when both senescence and age-dependent rates of resistance are accounted for.

More generally, since parasite evolution has received little attention using an age-structured framework, there is scope to include other traits that influence virulence evolution, which may depend on age. For example, in spatial models, higher host dispersal rates select for higher virulence (Boots & Sasaki 1999). In many organisms, older individuals are likely to disperse less than younger ones. Vaccination may also influence parasite evolution. Depending on the type of vaccine (infection blocking or parasite growth control), vaccination may increase or decrease parasite virulence (Gandon, Mackinnon, et al. 2001). Although in clinically relevant diseases vaccination age can be controlled, the administration of vaccines to free ranging wildlife is less precise. For example, oral rabies vaccines, which are administered in bait, may be received by individuals from many age classes or to a small part of a cohort only (Slate et al. 2005). It is well known that vaccination strategies can change the average age at infection, which can in some cases result in more severe disease outcomes, for example

congenital rubella syndrome (Anderson & May 1983), however the influence of vaccination age on virulence evolution is unknown.

6.3 Conclusion

This thesis demonstrates that taking account of host senescence can drastically influence the evolutionary outcome of host-parasite interactions. Moreover, our results provide a fresh perspective on how we should think about the transmission function, and the importance this has in the context of host population dynamics. We also show that the predictions of the classical theory of senescence evolution are robust in the context of host-parasite interactions. We consider this in the coevolutionary interactions of the host and parasite, and find that to some extent, it can be responsible for parasite driven extinction. We suggest there is considerable scope to develop the work here to take account of other age-dependent rates, such as resistance, which may provide variations on our predictions. Additionally, future experimental work should test our findings regarding senescence evolution in response to parasite infection. Our experimental results suggest that some of the predictions of coevolutionary models of tolerance, virulence and resistance, may in part be due to simplifying assumptions about the costs of defence. We hope that the work here encourages a deeper recognition of how coevolutionary interactions are determined by the genetic constraints in individual species.

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