Impact of environment on co-evolution between hosts and parasites

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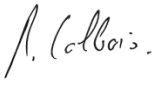

Abstract

Many parasites cause pathology and mortality and can be a major source of selection on their hosts, creating strong selection pressures on the evolution of hosts defense strategies. Changes in the host's tolerance and resistance to pathogen can strongly influence the spread of a disease and hence influence selection on virulence. Understanding how ecological conditions influence the host's life history and defense mechanisms, how they alter infection dynamics and contribute to the parasites virulence and transmission and how they shape the co-evolutionary dynamics is essential for gaining further insights into host-parasite interaction.

In this thesis, single generation experiments and experimental evolution were used to explore the impact of the environment on host-parasite interaction and their evolution. Firstly, the role of resource variability on the life history of the mosquito host *Ae. aegypti* was investigated. Secondly, the relationship between the growth of the parasite *V. culicis* and the health of its mosquito host was explored across ecological variables. The parasites growth rate and asymptotic load was estimated and compared in living and naturally dying hosts. Thirdly, theoretical predictions about the evolution of host defense against parasites were tested. In particular, the role of resource availability on the evolution of the host's tolerance and resistance to pathogen was investigated. Finally, this thesis examines the impact of the environment and co-evolving parasites on the host's life history evolution. The experiments introduced here show that variable environmental conditions can influence many central aspects of host-parasite interactions, ones that play important roles in shaping evolutionary dynamics. In this thesis, the results of those experiments are described in detail and their implications for host-parasite co-evolution are discussed.

Overall, this thesis emphasizes the complexity and dependence from environmental conditions of host-parasite interaction and their evolution. When considering the spatial and temporal ecological differences of natural habitats, the results presented here may help to lead to a more profound knowledge of host-parasite interactions.

Keywords

Aedes aegypti, co-evolution, epidemiology, experimental evolution, host-parasite interactions, life-history evolution, resource variability, *Vavraia culicis,* virulence

Résumé

Beaucoup de parasites provoquent des pathologies et de la mortalité et peuvent être une source majeure de sélection sur leur hôte, en appliquant une forte pression de sélection sur l'évolution de leurs stratégies de défense. Les changements de tolérance et de résistance de l'hôte face au pathogène peuvent fortement influencer la propagation d'une maladie, et donc influencer la sélection de la virulence. Comprendre comment les conditions écologiques influencent l'histoire de vie et les mécanismes de défense de l'hôte, comment elles altèrent la dynamique d'infection et contribuent à la virulence et la transmission du parasite, et comment elles façonnent la dynamique coévolutive est essentiel pour avoir une meilleure compréhension des interactions hôte-parasite.

Dans cette thèse, des expériences sur une seule génération et des expériences d'évolution expérimentale ont été utilisées pour explorer l'impact de l'environnement sur les interactions hôte-parasite et leur évolution. Premièrement, le rôle de la variabilité des ressources sur l'histoire de vie de l'hôte, le moustique *Ae. aegypti*, a été investigué. Deuxièmement, le lien entre la croissance du parasite *V. culicis* et la santée de son hôte moustique a été exploré sous différentes conditions écologiques. Troisièmement, le rôle de la disponibilité des ressources sur l'évolution de la tolérance et de la résistance de l'hôte au pathogène a été exploré. Finalement, l'impact de l'environnement et des parasites sur l'évolution des traits d'histoire de vie de l'hôte a été étudié. Les expériences introduites ici ont montré que des conditions environnementales variables peuvent influencer beaucoup d'aspects centraux des interactions hôte-parasite, en particulier ceux façonnant de manière importante la dynamique évolutive. Dans cette thèse, les résultats de ces expériences sont décrits en détail et leurs implications pour la coévolution hôte-parasite sont discutées.

Plus globalement, cette thèse souligne la complexité et la dépendance aux conditions environnementales des interactions hôte-parasite et leur évolution. En considérant les différences spatiales et temporelles des habitats naturels, les résultats présentés ici peuvent aider à conduire à une connaissance plus profonde des interactions hôte-parasite.

Mots-clés

Aedes aegypti, évolution de l'histoire de vie, co-évolution, épidemiologie, évolution experimentelle, interactions hôte-parasite, variabilité de resources, *Vavraia culicis,* virulence

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Chapter 1 General introduction

1.1 Background

Parasites[1] are omnipresent in nature an can be a substantial source of selection on their hosts. Parasites are involved in many ecological and evolutionary processes including the evolution of sex, social behavior, maintaining or increasing genetic diversity of hosts, altering population structures and even in the diversification of species. Some parasites are known to be highly virulent by causing serious harm and death in their host populations. For example, it has been estimated that 20% of the marine biomass is killed by viruses every day and that in the ocean 10^{23} new viral infections occur every second (Suttle 2007). There are more than 1,400 described parasite species that can infect humans (Woolhouse and Gowtage-Sequeria 2005) and approximately 15% of human death is caused by pathogens. Malaria alone accounts for 20% of childhood mortality and in 2015 there were an estimated 214 million new cases with 440,000 deaths (World Health Organization 2015). Other parasites are less severe but still cause debilitating symptoms, which can reduce or prevent reproduction and decrease competitive abilities.

Why some parasites cause very severe pathology and mortality while others are relatively benign, and why some parasites are very efficient in spreading in a population and become epidemic while others stay in small frequencies, has fascinated the scientific community for decades. Such diverse disease characteristics are the result of complex co-evolutionary dynamics between hosts and parasites. Parasites that cause pathology and mortality can be a major source of selection on their hosts. The hosts have evolved diverse mechanisms of defense to reduce the success of infection, increase the rate of clearance, or at least by reducing the detrimental effects of the parasite. These defense mechanisms range from very complex immune system processes, modification of cell surfaces to prevent infection, to changes in host behavior and life-history strategies in order to resist or tolerate parasites. The parasites on the other hand, are dependent on the host's resources and aim to reach a high density, within

[1] Parasites in thesis are defined broadly as infecitous agents that cause disease (including virus, bacteria, fungi, protozoa, helminths).

the host, in order to be transmitted efficiently in a population. However because a high parasite density is expected to reduce the host's survival and thus the duration of the transmission period, the parasites virulence[2] is traded-off with its transmission (Anderson and May 1982; Alizon et al. 2009).

Accordingly, the host's ability to resist parasites is in continuous conflict with the parasites infectivity, the growth and its transmissibility. An evolutionary response to parasitism of the host may again alter the selection pressure of the parasite. This reciprocal selection can result in co-evolution, with continuous changes of allele frequencies in hosts and parasites (Gandon et al. 2008; Gaba and Ebert 2009). This can be driven by parasite-mediated selection against common alleles or by directional selection through the successive fixation of advantages mutations. According to the red-queen hypothesis the evolutionary rates of change should be accelerated with coevolution. Indeed genomes of coevolving hosts can evolve faster compared to populations evolving against a constant parasite population (Paterson et al. 2010; Kashiwagi and Yomo 2011). However in many situations such predictions are too simple, especially when hosts develop tolerance instead of resistance, when there is enhanced competition for resources or when immunopathology causes a big part of the damage (Restif and Graham 2015). For example it has been shown that co-evolutionary dynamics can be altered by resource availability; high resource levels leads to decreased fluctuating selection in resistance (Lopez Pascua et al. 2014). Such studies illustrate the need to understand host-parasite interactions in an "eco-co-evolutionary" context because of the presence of genotype-by-genotype-by-environment interactions that influence their evolution.

Hosts resources affect many aspects of host parasite interaction and have shown to drastically change the outcome of infection (Shetty 2010). However, in most theoretical studies that predict the evolution of host-parasite interactions, one fundamental aspect of parasites is generally ignored: that parasites steal resources from their host to support their own development (see (Smith 1993; Hall et al. 2007; Hall et al. 2009a; Hall et al. 2010) for exceptions). In addition to genetic factors of hosts and parasites, resource availability has been recognized as a key aspect in the dynamics of infectious diseases, influencing host defense, parasite transmission and virulence (Lazzaro and Little 2009; Wolinska and King 2009). Several studies show how resource quality, as well as quantity, shapes parasite virulence (Jokela et al. 1999; Brown et al. 2000; Ferguson and Read 2002) and also directly influences the production of parasites (Bedhomme et al. 2004; Johnson et al. 2007; De Roode et al. 2008; Hall et al. 2009b).

[2] Virulence in this thesis is definded as the parasite induced host mortality and fecundity loss from the age of infection onwards.

There are two general ways in which resource availability in the host's environment influences host-parasite interactions. On the one hand, high levels of resources can positively influence the host's immune response and therefore increase its resistance to parasite infection (Koella and L. 2002; Ayres and Schneider 2009). Malnourished hosts maybe weaker and more susceptible to infectious disease (Moret 2000) so that the lower the food availability, the higher the costs of parasitism (Ferguson and Read 2002). For example, the flea *Xenopsylla ramensis* produces more eggs when feeding on hosts experiencing diet restriction (Krasnov et al. 2005). On the other hand, a parasite uses the host's internal resources for its own development. Accordingly, hosts reared on high levels of food may store more energy reserves and therefore present a better environment for the parasite to develop. Indeed, enhanced qualities and quantities of resources increase the production of effective propagules of parasites (e.g. ascogregarines (Tseng 2006) or microsporidians (Agnew and Koella 1999b; Bedhomme et al. 2004)) in mosquitoes, and trypanosomes in bumble-bees (Brown et al. 2000), but also increase host survival (as in (Jokela et al. 1999)), and can negatively affect host reproductive success. Well-fed *daphnia* hosts infected with *Caullerya mesnili*, for example, showed, in regard to fecundity, more severe parasitic effects (Bittner et al. 2002). It further has been shown that increased food availability increases parasite transmission rate (Ebert et al. 2000; J. Ryder et al. 2007; Vale et al. 2013).

Because parasite growth, virulence and transmission are principal parameters in epidemiology and the evolution of host-parasite interactions, ignoring the impact of resource availability limits our understanding of host-parasite interactions and evolution. Studying these traits under different resource levels is therefore vital to understand the parasites within host-dynamics (e.g. the relationship between parasite burden and host health), which plays an important role in the evolution of parasites and hosts (Antia et al. 1994; Alizon and van Baalen 2005a). By considering that the host's natural habitat can vary drastically in space and time, our understanding of host-parasite interactions, their co-evolution, the predictions of parasite evolution and especially the management of parasites must incorporate knowledge of how environmental variations influence parasite-host interactions. It is therefore of great importance to science and society to deepen our knowledge in this field.

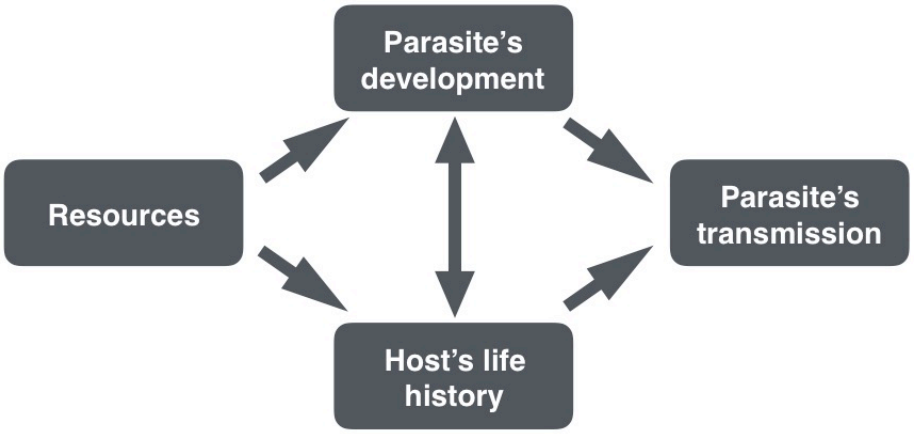

Figure 1:1 Resources in host-parasite interaction

Resource availability as a key factor in host-parasite interactions: Resource availability can influence the host's growth rate, age and size at maturity, longevity and fecundity. Resources can also be important for the development of the parasite within its host because of a direct influence of the resources available to the parasite, and via the hosts life history, in particular body size that often constrains the parasite development. The amount of resources can also influence the host's resistance (limit parasite development) and tolerance (reduce detrimental effects of parasites without affecting the parasites development) to pathogens. The development of the parasite can feedback onto the host's life history, especially by reducing its longevity. The transmission of the parasite is dependent on parasite growth and the longevity of the host, and indirectly on the host's resources.

1.2 Thesis introduction

In this thesis I study the impact of the environment on host-parasite interactions (summarized in Figure 1.1) and how this influences their evolution. I use the mosquito *Aedes aegypti* and its microsporidian parasite *Vavraia culicis*. I aim to empirically test certain aspects of mathematical models that predict the evolution of the host and parasites life-history, virulence and the parasites defense strategies. These results will hopefully enlarge our knowledge about the evolution of host parasite interactions in general, and contribute to the development of more powerful and realistic life-history and epidemiological models. Furthermore, the data might be directly relevant for public health because of the role of mosquitos as a vector for several infectious diseases. The results might also be useful for people working on biological control as microsporidians have been proposed for the control of mosquitoes and their vector-borne diseases (Sweeney and Becnel 1991; Koella et al. 2009; Lorenz and Koella 2011).

1.2.1 Food variability, growth and later life consequences

Resource availability is as key component of life history theory because of its role in determining growth, survival and fecundity of individuals (Stearns 1992). Diet restriction has been associated with

slower growth, smaller adult size, delayed maturity and lower fecundity, but with a longer and healthier life (Stearns and Koella 1986). Because adult size is a very important determinant of fitness, adaptations to handle periods of food restrictions are very important. One possibility, which is referred to as compensatory growth, is to grow at accelerated rates after a period of undernourishment in order to catch up in size. Rapidly growing individuals might respond to food stress by decelerating growth rates in order to use the available resources for maintenance and reproduction. Compensatory growth, as well as decelerating growth, are generally thought of as adaptive responses, but their consequences later in life remain largely unexplored. In the second chapter, I experimentally test the effect of variable food availability during the development of the mosquito host *Aedes aegypti* (leading to compensatory growth or decelerated growth), and the associated changes in longevity and reproductive success. Such data is relevant for life-history theory and is also directly relevant for public health due to the mosquito's role as vector for several infectious diseases.

1.2.2 Within-host growth and virulence

Most models for the evolution of host parasite interactions are based on the assumption that there is trade-off between virulence and the rate of transmission (Anderson and May 1979; Alizon and Lion 2011, Gandon et al. 2001; Dieckmann et al. 2002). Underlying this assumption is the idea that parasites must grow rapidly to a high load in order to be transmitted efficiently, but that high parasite density also reduces the host's survival and thus the duration of the transmission period. This trade-off implies that maximal transmission is often highest at intermediate levels of virulence. However optimal levels of virulence depend on the shape of the trade-off (Anderson and May 1982; Bremermann and Pickering 1983). The general assumption that parasite burden is positively correlated with its virulence holds in several systems (Mellors et al. 1996; Mackinnon and Read 1999; De Roode et al. 2008; De Roode and Altizer 2010). However, because resource availability has been shown to influence parasite growth (Johnson et al. 2007; De Roode et al. 2008; Michalakis et al. 2008; Hall et al. 2009b) and virulence (Jokela et al. 1999; Brown et al. 2000; Ferguson and Read 2002), it is likely to affect the association between the two, so that we only see a negative relationship between parasite development and longevity in some environments. In the third chapter I investigate experimentally how aspects of the environment (the amount of food available to larvae and to adults, and the age at infection) influences the growth of the parasite, the longevity of the host and the relationship between the two. Studying factors that influence the relationship between the host's growth and its virulence is important, because they may alter the relative costs and benefits of rapid parasite replication and therefore determine adaptive levels of virulence.

1.2.3 Tolerance and resistance: two fundamentally different defense traits

Adaptations of the host to reduce negative effects of parasites can be classified into two broad categories: Resistance and tolerance to parasites (Read et al. 2008; Råberg et al. 2009; Little et al. 2010). Resistance reduces the success of infection or increases the rate of clearance, whereas tolerance reduces the detrimental effects of the parasite without affecting the pathogen directly. It is important to distinguish between the two traits because they have a very different effect on the evolution of host-parasite interactions (Roy and Kirchner 2000; Restif and Koella 2003; Boots 2008). Tolerance genes are predicted to become fixed, because they are beneficial for the host and parasite, while resistance genes are predicted to rapidly change because they would provoke counter-adaptation of the parasite to overcome resistance (Roy and Kirchner 2000; Miller et al. 2006). One example that illustrates the evolutionary significance of tolerance is the case of simian immunodeficiency virus (SIV) in monkeys. In macaques, which are non-natural hosts, the virus replicates rapidly and infected animals develop AIDS. In contrast, in sooty mangabeys, which are natural hosts of SIV, infected individuals do not show disease preogression and show no development of AIDS, even when carrying high virus loads (Chakrabarti 2004). Sooty mangabeys, which have a long evolutionary history with SIV, therefore reduced the damage of parasite infection and evolved high levels of tolerance instead of resistance. Tolerance has also been discussed to be clinically relevant (Medzhitov et al. 2012). In contrast to resistant-based therapy, tolerance-based therapy aims to improve the health of the host at a given parasite load instead of reducing the parasite burden. This may not lead to selection for resistant parasites and was considered as “evolution-proof” (Read et al. 2008; Schneider and Ayres 2008).

Differentiating between resistance and tolerance, studying the association between the two, and predicting how the two evolve have become important topics of evolutionary parasitology. What is missing are experimental studies on the extent to which evolution favors tolerance or resistance under different ecological settings. Using experimental evolution, I aim to test theoretical predictions about the evolution of host defense against parasites and investigate the role of resource availability on the concurrent evolution of tolerance and resistance.

1.2.4 Change in life history to reduce the costs of parasitism

A change in host’s life history can be another form to reduce the cost of parasitism. Life history theory predicts that early maturing hosts may have a selective advantage because they can evade parasitism in time and when parasitized reduce detrimental effects on reproduction and longevity (Hochberg et al. 1992; Forbes 1993; Perrin and Christe 1996). However such adaptations can be associated with costs later in life (reduced late-life fecundity and longevity). A stunning example is the spread of an infectious

cancer in Tasmanian devils that causes almost complete mortality after the first year of adulthood. This facial tumor causes an abrupt shift in the host's life history from multi breeding toward single breeding. The Tasmanian devils responds to this strong selection pressure, by a 16-fold increase in precocious sexual maturity (Jones et al. 2008). Such an alteration in life history can be seen as a form of resistance, which might lead to complex co-evolutionary dynamics between the life histories of the host and the parasites. In the fifth chapter I aim to study how coevolving and constant parasites can influence the hosts' life history under different resource levels. Studying the role of resource availability is potentially relevant because trade-offs between different life history traits might only be detectable when resources are scarce. Accordingly, variable environments might influence the long-term host evolution.

1.3 Experimental system

In this thesis the mosquito species *Aedes aegypti* and its microsporidian parasite *Vavraia culicis* were used as model organisms to investigate the impact of the host environment on host-parasite interaction.

1.3.1 The mosquito *Aedes aegyti*

The UGAL strain of the mosquito species *Ae. aegypti* (obtained from Patrick Guérin, University of Neuchâtel) was used for all of the experiments presented in this thesis. The mosquito species *Aedes aegypti* is the principal vector of yellow-fever (Tomori 2004), dengue (World Health Organisation 2002) and Zika-virus (Petersen et al. 2016), grows in a variety of different natural and artificial containers holding clean fresh water (Southwood et al. 1972) and occurs throughout the tropics and subtropics. It is a very well studied species; its ecology is known in detail (Christophers 1960), it has been a model organism in insect physiology studies (Clements 1999), and its full genome has been published (Nene et al. 2007). *Aedes aegypti* are highly susceptible to a whole range of environmental conditions (Christophers 1960). For example during the aquatic larval stages, wild mosquitoes are often undergoing periods of nutrient restriction and competition for resources like bacteria, algae and organic matter (Reiskind and Lounibos 2009).

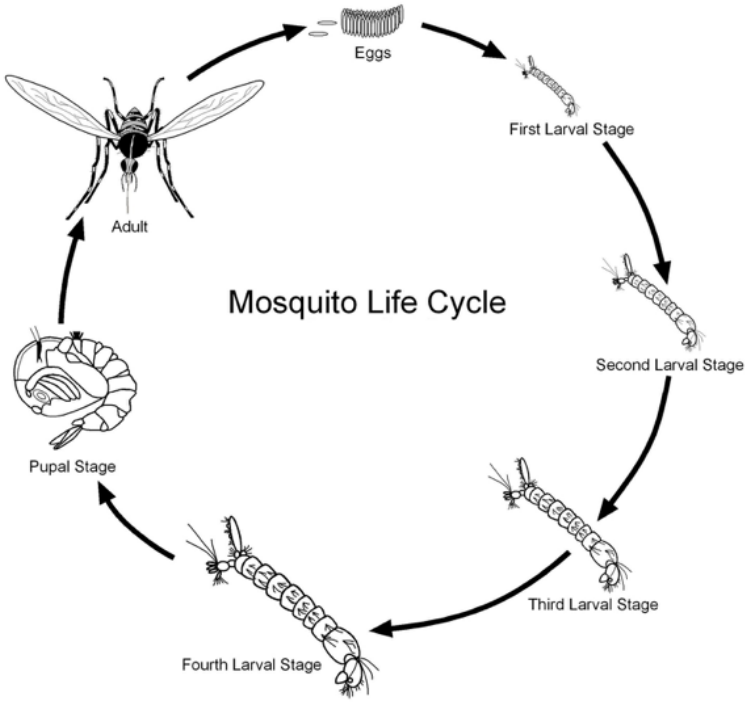

Figure 1:2 Mosquito life cycle.

Generalized life cycle of mosquitoes: *Aedes aegypti* mosquitoes are holometabolous organisms. Each mosquito goes through four distinct stages of its life cycle: Egg, larva, pupae and imago. The adulthood is the only non-aquatic stage.

1.3.2 Vavraia culicis

Microsporidia constitute a large, very diverse group of single-cell, endocellular parasites. They belong to the kingdom of fungus, are widespread in nature and have a broad range of hosts (from humans to invertebrates). Microsporidia are specially common in arthropods; close to half of the described genera have insects as their hosts (Becnel and Andreadis 1999). For this thesis I use the microsporidan parasite *Vavraia culicis* that was originally derived from *Ae.albopictus* in Florida and kindly provided by J.J Becnel (USDA, Gainesville, USA). It is a natural parasite of several genera of mosquitoes including *Aedes aegypti* (Weiser and Coluzzi 1972). Natural prevalence rates of infection ranging from less than 1% up to 54% differing from mosquito species and geographical location (Andreadis 2007). It is an obligate endocellular parasite, orally infectious and is transmitted between hosts by infective spores (Figure 1.3). The spores are they only stage of the parasite that can persist in the environment outside the host cell. The host larvae ingest the spores of *Vavraia culicis* with their food, resulting in infection of gut cells and epithelial cells. After passing a series of developmental stages within the larvae, the parasite begins to produce its infectious spores. The infection then spreads to gut and fat body cells. No component of an insect's immune system has been described which can neutralize this intracellular parasite (Biron et al.

2005). The parasite is transmitted in two ways. First, transmission can occur from larva to larva after larval death. In particular cases of food stress or strong infection (Bedhomme et al. 2004) larval death is enhanced, which initiates another round of horizontal transmission. Second, larvae survive the infection, without clearing it and develop into adults. The spores within the host contributes to the parasites fitness either by the release of spores when dying in the aquatic environment or, because spores can adhere to the surface of the eggs and infect the newly hatched larvae (Andreadis 2007).

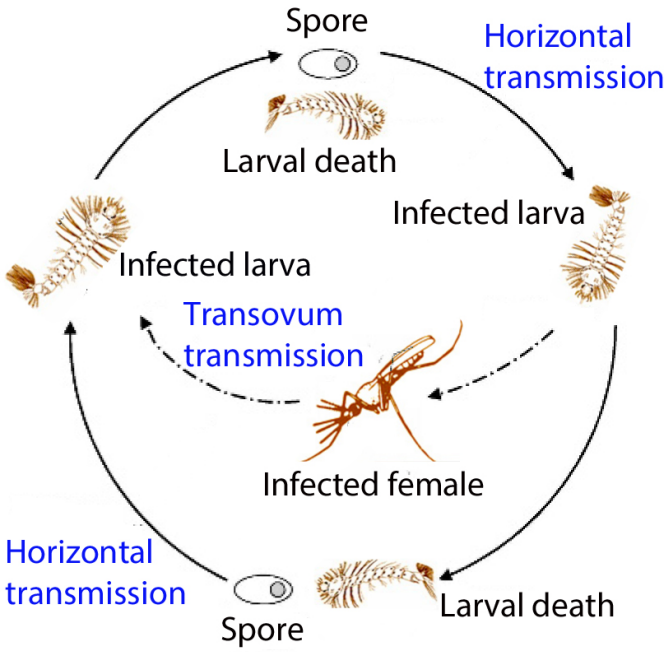

Figure 1:3 Life cycle of *Vavraia culicis*

Life cycle of *Vavraia culicis*: Spores are released into the aquatic environment when juvenile or adult mosquitoes carrying spores die and are orally ingested by larvae. Transmission can occur from larva to larva when spores are released after the larva dies (solid lines) or if larvae survive the infection and develop into adults; the spores can be released when the mosquito dies in the aquatic environment or they can adhere to the surface of the eggs and infect the newly hatched larvae (dashed lines).

1.3.3 Resources affect life-histories of host and parasite

Resource availability plays a fundamental role in the interaction between mosquitoes and microsporidian parasites. Larval resource availability of the host influences the mosquito's growth rate, age at ma-

turity, reproduction and longevity (Zeller and Koella 2016) as well as it influences the development of the parasite by increasing the production of infectious spores of dead mosquitoes (Bedhomme et al. 2004). The within growth of the parasite may therefore be limited by the condition of the host, probably because of a direct influence of the energy available for the parasite (Hall et al. 2009b) and an indirect impact via the host's life history, specially body size, that constrains the development of the parasite (Agnew and Koella 1999b). Larval food also determines the virulence of the parasite: less food enhances juvenile mortality (Bedhomme et al. 2004; Lorenz and Koella 2011) and decreases adult lifespan of infected mosquitoes (Lorenz and Koella 2011). It also considerably affects the parasite's transmission route, either horizontally from dead juveniles or vertically from females when the lay their eggs.

1.4 Research aims

The overall object of this PhD thesis is to understand empirically how the environment influences the co-evolutionary dynamics between the mosquito *Aedes aegypti* and its microsporidian parasite *Vavraia culicis*. I aim to empirically test assumptions and predictions of models of host and parasites life-history and epidemiology. I use single generation experiments as well as experimental evolution to address the open questions.

The project has four major goals:

I. Experimental examination of resource variability on growth, reproduction and longevity in the mosquito *Aedes aegypti*
II. Description of the parasites within-host dynamics; investigation of the relationship between parasite development and host-longevity as a function of the hosts resources and age at infection
III. Test theoretical predictions about the evolution of host defense against parasites. Investigation of the role of resource availability on the evolution of the host's ability to tolerate and resist parasites.
IV. Examination of resource availability and co-evolutionary dynamics on the host life-history evolution.

Chapter 2 Effects of food variability on growth and reproduction of Aedes aegypti

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Abstract

Despite a large body of knowledge about the evolution of life-histories, we know little about how variable food availability during an individual's development affect its life-history. We measured the effects of manipulating food levels during early and late larval development of the mosquito *Aedes aegypti* on its growth rate, life-history and reproductive success. Switching from low to high food led to compensatory growth: individuals grew more rapidly during late larval development and emerged at a size close to that of mosquitoes consistently reared at high food. However, switching to high food had very little effect on longevity, and fecundity and reproductive success were considerably lower than in consistently well fed mosquitoes. Changing from high to low food led to adults with similar size as in consistently badly nourished mosquitoes, but even lower fecundity and reproductive success. A rapid response of growth to changing resources can thus have unexpected effects in later life and in lifetime reproductive success. More generally, our study emphasizes the importance of varying developmental conditions for the evolutionary pressures underlying life-history evolution.

2.1 Introduction

How life-histories respond to variation in food availability is a central question of evolutionary ecology. Considerable effort, both with theoretical and empirical approaches, has been spent on answering the question for environments that vary spatially (Kawecki and Stearns 1993; Ernande et al. 2004) and from one generation to the next (Bashey 2006) in resource availability. Yet, an important aspect of variability has received considerably less attention: that resource levels can vary during an individual's development.

Even though there is substantial evidence that variation in food levels during development can affect age and size at maturity (e.g. Leips & Travis 1994; Hentschel & Emlet 2000), we know little about how this variation affects reproductive success and adult survival. As food restriction severely affects life-history parameters - it generally slows growth, delays maturity and leads to small adults with low fecundity (Stearns and Koella 1986) - it seems plausible that individuals that grow slowly early in life should try to make up their size deficit with compensatory growth, i.e. by growing more rapidly or for a longer period once they obtain more food (Dmitriew 2011). Rapidly growing individuals, on the other hand, might respond to food stress by decelerating growth rates in order to use the available resources for maintenance and reproduction. Slow growth has shown to be adaptive for dealing with nutrient stress (Arendt 1997).Compensatory growth following a period of unfavorable environmental conditions has been described for many vertebrates and invertebrates (Dmitriew 2011). However, compensatory growth need not be evolutionarily beneficial. Indeed, the presumed benefit of compensatory growth - larger individuals have greater fecundity - is not always observed. In Trinidadian guppies, for example, compensatory growth is not associated with increased, but with decreased fecundity (Auer et al. 2010). Furthermore, any benefit of compensatory growth with regard to fecundity may be counteracted by costs with regard to other parts of the life-history. Longer growth and thus delayed maturity, for example, can be associated with a greater risk of dying before maturity (Abrams and Rowe 1996). Even for pure compensatory growth, i.e. when maturity is not delayed, the greater growth rate may have costs (physiological/cellular level), which are often only evident much later in life (Metcalfe and Monaghan 2001; Alonso-Alvarez et al. 2007; De Block and Stoks 2008). Indeed, diet restriction is often associated with a longer and healthier life (Chippindale et al. 1993; Masoro 2005). Accordingly compensatory growth in fish reduced lifespan whereas decelerated growth extended it (Lee et al. 2013). This may, in part, be due to developmental errors and structural instability as a result of increased growth (Mangel and Munch 2005). Thus, although the role of re-feeding after a period of dietary restriction (and, more generally, the role of changing resource availability during an individuals' development) on traits such

as growth rate, longevity and age at maturity have acquired some attention, little is known about its role on reproductive success.

In this study, we provide data on the effect of variability in developmental food conditions (leading to compensatory growth or decelerated growth) and associated changes in longevity and reproductive success of the mosquito *Aedes aegypti*. Such data not only form the basis for our understanding of life-history evolution, but are also directly relevant for public health due to mosquito's role as a vector of several infectious diseases.

2.2 Material and methods

2.2.1 Experimental system

We used the UGAL strain of the mosquito *Ae. aegypti* (obtained from Patrick Guérin, University of Neuchâtel). *Aedes aegypti* occurs throughout the tropics and subtropics. During the aquatic larval stages, mosquitoes in nature can experience periods of nutrient restriction and competition for resources like bacteria, algae and organic matter (Reiskind and Lounibos 2009).

2.2.2 Experimental design

The experiment was run in a climate chamber set to 26° C, 70% relative humidity and at 12h light and 12h dark regime. We used a 2x2 factorial design, where larvae were fed either with a standard amount of food (Day 1: 0.06mg of tetramin fish food, day 2: 0.08mg, day 3: 0.16mg, day4: 0.32mg, day 5: 0.64mg, day 6 or later: 0.32mg) or with half of the standard diet during either early (0 to 3 days after hatching) or late development (4 or more days after hatching). The four treatments are hereafter referred to as LL, LH, HH and HL, with the first letter referring to the amount of food during early development (Low or High) and the second letter to the amount of food during late development. Eggs were hatched in deionized water. Four hours after hatching, 384 first-instar larvae were moved into 12-well plates and kept individually in 3 ml of deionized water. Each larva was haphazardly assigned to one of the four feeding regimes and fed every 24 hours with the appropriate amount of food. Pupae were moved to 300ml plastic cups containing deionized water and a piece of filter paper as an oviposition substrate. The cups were covered with mosquito netting, and cotton wool moistened with 10% sugar solution was placed onto the netting and changed every 48 hours. One day after emergence, males were discarded and each female was given a male chosen haphazardly from our colony. The next day and every ten days thereafter, the females were given the opportunity to take a blood meal on MZ's

arm for five minutes. The females where checked every day for survival. Nine days after blood feeding, the females were placed into freshly prepared plastic cups and their eggs were removed and counted. Fecundity was defined as the number of melanised eggs laid up to nine days after blood feeding. The experiment was stopped after six rounds of egg-laying, at which time 85.4 % of the mosquitoes had died.

2.2.3 Trait measurement

We estimated larval body size by taking standardized digital pictures of all individuals every 24 hours starting on the day of hatching (age 0) and measuring the length of the larva with the open-access software IMAGEJ. When photos of larvae were considered too low in quality for an accurate measurement to be taken, the individuals were not included in the analyses. Larval growth was measured as the difference in size between age 0 and age 4 (early growth) and between age 4 and age 6 (late growth) for all individuals. The size of adults was assayed as the mean of their wing length, which strongly correlates with the weight of mosquitoes (Koella and Lyimo 1996) and is widely used as an approximation for adult size. The wings were removed and mounted on microscope slides. The slides were digitally scanned and the wings were measured with IMAGEJ

2.2.4 Statistical analysis

We considered only females, and ignored the growth of the 6 (out of 384) individuals that had died before pupation. We assayed 185 female mosquitoes, between 43 and 49 in each food treatment. The difference in size between age 0 and age 4 (early growth) was evaluated with an analysis of variance (ANOVA) that included the level of early food as a fixed binomial factor. Because the size differences between the ages 4 and 6 (late growth) were close to linear and individuals not yet reached asymptotic size they were evaluated with an analysis of covariance (ANCOVA) that included early and late food, the interaction between the two as fixed factors, and the size at age four as a covariate. As size at age four did not interact with early or late food, we omitted these interactions from the analysis. Additionally, because we measured individuals repeatedly, we checked that the results were similar, when we corrected for regression to the mean (analysis not shown). For both analyses (early and late growth) we verified that the assumptions of ANOVA and respectively ANCOVA were not violated. Age at emergence and longevity were analyzed with survival analyses that included early and late food and their interaction as fixed factors. In the analysis of longevity we added wing length as a potential confounder. We used the distributions that gave the best fit, so log-logistic for age at emergence and Weibull for longevity; using proportional hazards gave similar results (not shown). Wing length was analyzed with an ANOVA that included early food and late food and their interaction as fixed factors. The wing lengths

were Box-Cox transformed to meet ANOVA requirements. We analyzed fecundity in three ways. First, we analyzed the proportion of blood-feeds that led to at least one egg with a GLM (binomial distribution). Second, we analyzed the total number of eggs laid throughout the experiment with a GLM with quasi-Poisson distribution (corrected for overdispersion). In both analyses, we included early and late food and their interaction as fixed factors and wing length as a potential confounder. Third, we analyzed the age-specific clutch sizes (considering only those blood-feeds after which at least one egg had been laid) with a mixed effect ANOVA, using early food, late food, clutch number (i.e. age) and their interactions as fixed factors, wing length as a potential confounder, and mosquito as a random effect. We present the analysis using all clutches. As the number of mosquitoes surviving to the end of the experiment was low, we verified that the results were similar if we considered only the first three or the first four clutches (analyses not shown). The mixed-effect ANOVA was done with R v.0.98.1056 (R Development Core Team, 2015) using the lme4 package; the other analyses were done with JMP 12.0.0.

2.3 Results

2.3.1 Developmental traits

The growth data are summarized in Fig. 2.1. Larvae reared on high food grew more between age 0 and age 4 (mean = 2.64 mm, standard error = 0.072) than those reared on low food (mean = 1.86 mm, se = 0.063) ($F = 66.86$, $p<0.001$) (Fig. 2.1). Growth after age 4 decreased with increasing size at day 4 (Table 2.1). It was greatest for mosquitoes that switched from low to high food at age 4 (2.21mm, se = 0.120), lowest for mosquitoes that had switched from high to low food (1.57mm, se = 0.118) and intermediate for mosquitoes with the same food level throughout their development (Fig. 2.2b). The effects of early and of late food, but not the interaction between the two, were statistically significant (Table 2.1).

Table 2:1 Statistical summary for juvenile traits

ANCOVA for differences in late growth, survival analysis (log Logistic distribution) for age at emergence and ANOVA for differences in wing length.

	Late growth				Age at emergence			Wing length			
Factor	df	F	SS	p	df	χ^2	p	df	F	SS	p
Early food	1	5.07	1.77	0.026	1	173.6	<0.001	1	2.84	0.08	0.094
Late food	1	14.87	5.18	<0.001	1	25.5	<0.001	1	41.21	0.02	<0.001
Early food * late food	1	0.24	0.08	0.63	1	6.6	0.01	1	0.13	<0.01	0.721
Size at age 4	1	90.62	31.57	<0.001							
Error	155		52.61					166		4.64	

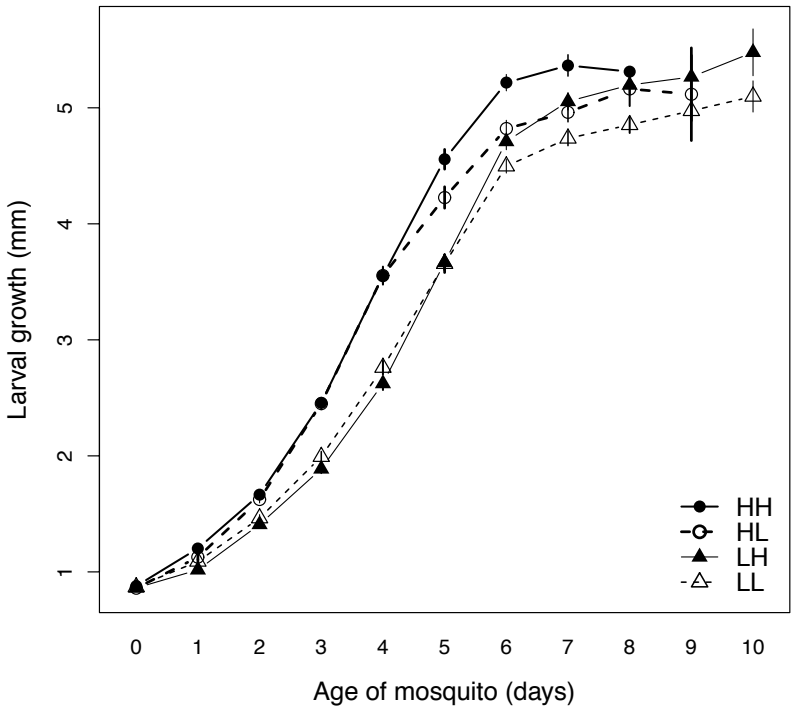

Figure 2:1 Mosquito growth

Body length for mosquito larvae as a function of age. Symbols represent the means with each food treatment, vertical lines the standard errors. Triangles represent treatments with low food availability during early development; circles represent treatments with high food availability in early development. Open symbols represent treatments with low food during late development; solid symbols represent high food during late development.

Age at emergence increased from 9.9 days (se = 0.11) for mosquitoes consistently fed the high food level to 11.9 days (se = 0.09) for mosquitoes consistently fed the low food level (Fig. 2.2d). Mosquitoes that had switched from high to low food emerged earlier (10.1 ± 0.09) than those that had switched from low to high food (11.2 ± 0.10); the interaction between early and late food levels was statistically significant (Table1, Fig. 2.2c).

Wing length increased from a mean of 2.35 mm (se = 0.019) for mosquitoes that had been consistently reared on low food to 2.55 mm (se = 0.027) for mosquitoes that had been consistently reared on high food. Wing length was influenced significantly by the availability of food after age 4, while early food and the interaction between early and late food had no significant effects (Table 1, Fig. 2.2d).

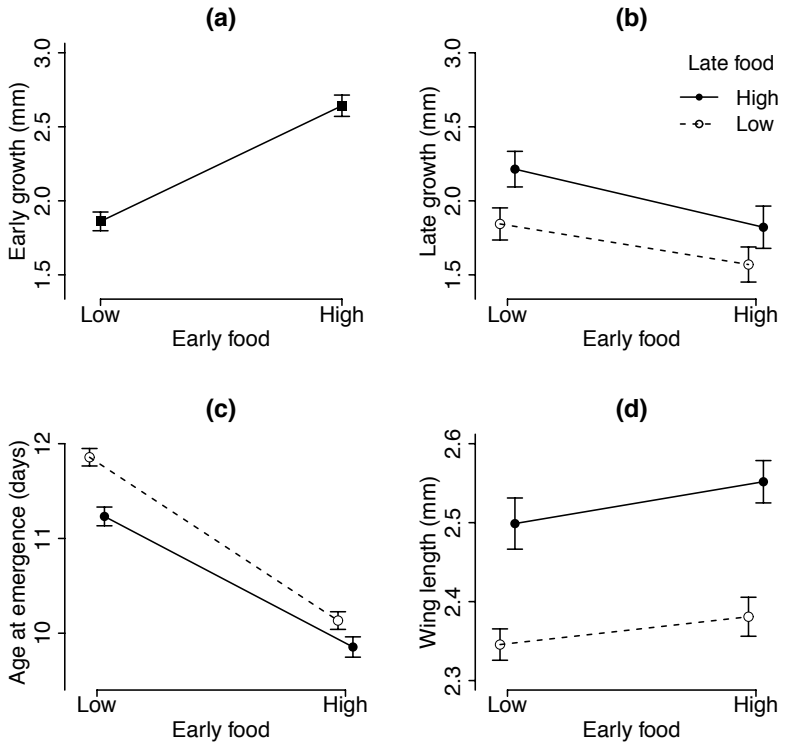

Figure 2:2 Juvenile traits

The effect of larval food during early and late stages of development for (a) Early growth (size difference between age 0 and age 4), (b) Mean late growth (size difference between day 4 and day 6), (c) Age at emergence ±SE, (d) Adult size (wing length). The data for early growth (a) was pooled for late food treatment. Symbols represent the means within treatments; the vertical lines their standard errors. Open symbols represent treatments with low food during late development; solid symbols represent high food during late development.

Table 2:2 Statistical summary for adult traits

Survival analyses (Weibull distribution) for longevity, binomial GLM for the proportion of blood-feds and GLM (quasi-Poisson distribution) for the total number of eggs.

		Longevity		Egg-laying after blood feeding		Total number of eggs	
Factor	df	χ^2	p	χ^2	p	χ^2	p
Early food	1	3.87	0.049	0.74	0.39	0.03	0.857
Late food	1	0.22	0.636	4.58	0.032	11.79	<0.001
Early food * late food	1	<0.01	0.969	4.00	0.046	3.66	0.055
Wing length	1	1.17	0.279	0.14	0.712	0.06	0.803

2.3.2 Adult traits

Adult mosquitoes lived longest if they had been reared on low food throughout their development (39.1 days ± 1.97; this and other averages are biased, for the experiment was stopped when 14.6% of the mosquitoes were still alive), followed by those that had switched from low food to high food when they were four days old (36.8 days ± 2.44). In contrast to the size of adult mosquitoes, longevity was significantly affected by early food (Table 2.2), while late food and the interaction between the two food levels had no significant effects. Wing length had no significant effect on longevity (Table 2.2).

The percentage of the six blood-feeds that were followed by laying at least one egg ranged from 0% to 100%; the average percentage ranged from 50% for the mosquitoes that had been reared on high food throughout their development to 28% if the mosquitoes had switched from high food to low food when they were four days old (Fig. 2.3B). About 35% of the blood-feeds led to egg-laying, if mosquitoes had initially been reared on low food, independently of the food available to them during their late development (Table 2.2). Similarly, the total number of eggs was highest for mosquitoes that had been reared on high food throughout their development (67 ± 8.1), lowest for mosquitoes that had switched food from high to low (31 ± 5.2) and intermediate for mosquitoes that had been reared on low food early in their development (for LL: 38 ± 4.7; for LH: 48 ± 6.9) (Table 2, Fig. 2.3C). Late food environments had significant effects in determining the probability of laying eggs and the total number of eggs. The interaction between early food and late food had marginally significant effects in determining egg-laying success and marginally non-significant effects in determining the total amount of eggs. Neither the egg-laying success nor the number of eggs were significantly influenced by wing length (Table 2.2).

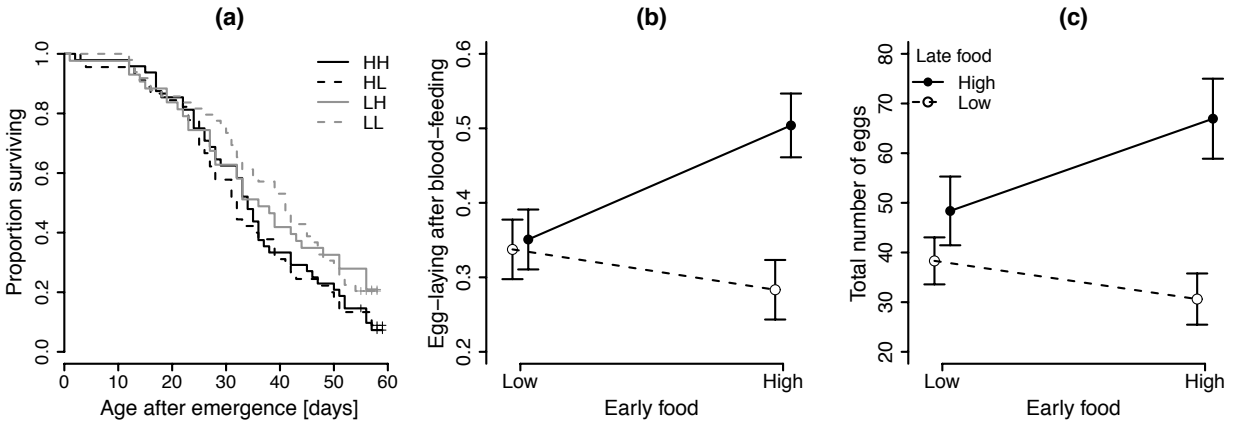

Figure 2:3 Adult traits

(a) The effect of larval food during early and late stages of development for (a) Longevity of adult female mosquitoes (age 0 is age after emergence). (b) Proportion of blood-feds that led to egg-laying, (c) Total number of eggs ±SE. LL stands for low food availability during the whole larval development, LH for low food during early development, high food during late development, HH for high during the whole development and HL for high food during early development, low food during late development. In (b) and (c), open symbols represent treatments with low food during late development; solid symbols represent high food during late development.

The clutch size (considering only those blood-feeds after which at least one egg had been laid) decreased with the age of adult mosquitoes (Fig. 2.4). Food level during late larval life affected the number of eggs in the first clutch and the rate at which fecundity decreased with age was influenced by the interaction between early and late food treatment (Table 2.3). Switching from low food to high food four days after hatch led to the most eggs in the first clutch, but then to the greatest decline over clutches (Fig. 2.4). The rate of the decrease was mostly influenced by the interaction between early and late food treatments (Table 2.3).

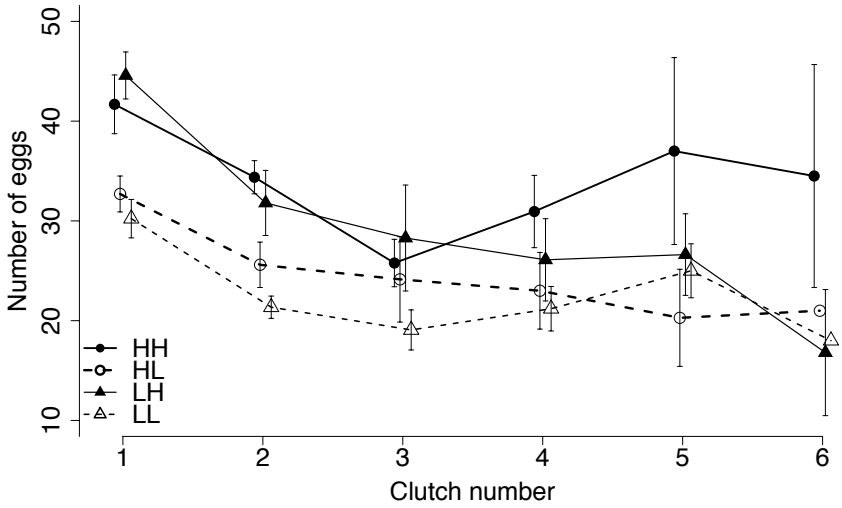

Figure 2:4 Clutch size

Relationship between number of eggs per clutch and clutch number (i.e., age). Circles represent treatments with high food availability during early development; triangles represent treatments with low food availability in early development. Open symbols represent treatments with low food during late development; solid symbols represent high food during late development.

Table 2:3 Repeated measures analysis of clutch sizes

Only blood-feeding attempts, which led to at least one egg, were considered.

	Number of eggs			
Factor	df	F	SS	p
Early food	1	0.02	2.8	0.885
Late food	1	15.00	2005.0	< 0.001
Early food * late food	1	3.67	490.6	0.056
Wing length	1	0.15	19.8	0.700
Clutch number	1	33.81	4518.5	< 0.001
Clutch number * early food	1	0.29	38.3	0.593
Clutch number * late food	1	0.61	81.8	0.435
Clutch number * early food * late food	1	5.52	737.6	0.020
Error	267		133.6	

2.4 Discussion

Variability in developmental food conditions in Aedes aegypti had qualitatively different effects on the life-history traits we investigated: adult size, fecundity, survival and reproductive success. Thus, for example, wing length was determined mainly by food availability during late larval development, survival by food availability during early development, and total number of eggs by a combination of the two.

When food availability was held constant during the mosquitoes' development, their life-history followed the general predictions of life-history theory (e.g. Stearns and Koella, 1986): low food thus led to slow growth, late pupation, small adults, and low fecundity. It also corroborates many studies where food restriction increased longevity (Weindruch 1996; Shanley and Kirkwood 2000; Mair et al. 2003; Kirkwood and Shanley 2005; Masoro 2005).

Varying food availability led to life-histories that are more difficult to explain with life-history, similarly to the study of Yearsley, Kyriazakis & Gordon (2004). Increasing from low to high food led, as frequently observed (Metcalfe and Monaghan 2001), to compensatory growth: at emergence, mosquitoes that had been first badly and then well nourished caught up in size by growing more rapidly and by delaying pupation, and thereby became almost as large as mosquitoes that had been fed well throughout their development. However, although size caught up, we observed no to very little catching up of fecundity, longevity, or life-time reproductive success. Together with the observation that the number of eggs per clutch declined strongest with age for individuals that had switched from low to high food during development (Figure 2.4), these results could mean that compensatory growth early in life is associated with reproductive costs later in life, which lead to, in our laboratory conditions, lower life-time reproductive success. In addition to considerable evidence for trade-offs between life-history traits early and late in life, both from laboratory situations (e.g. Rose 1984) and, more recently, from natural populations (Lemaître et al. 2015), our results support the findings of Auer et al. (2010), which suggest that there are reproductive costs associated with compensatory growth. The trade-off we observed raises the question about the adaptive nature of compensatory growth. However, although in our laboratory conditions, compensatory growth had a negative consequence for reproductive success, the situation may change in natural conditions. Both juvenile and adult mortality rates may be substantially higher in the field than in the laboratory. Accordingly the benefits of larger size and earlier maturity associated with compensatory growth may outweigh its reproductive costs in old mosquitoes.

When mosquitoes started out at good food conditions and then switched to low food, their growth and adult size decreased as expected. What was more surprising was that the individuals with decelerated

growth have lower reproductive success than those that had experienced food restriction throughout their development. However, because the interaction between early and late food was marginally not significant, we cannot draw strong conclusions. Nevertheless this trend could be the result of physiological responses to the food environment in early development that prepare the individual for a similar environment later in life (Gluckman and Hanson 2004). Therefore, mosquitoes that are undernourished early in life can cope with food restriction later in life better than those that have been prepared for an environment with plentiful food.

A striking result was that wing length had very little effect on reproduction or longevity, although associations of life-history traits with size are central to many ideas in life-history theory (e.g. Stearns & Koella 1986; Rowe & Ludwig 1991; Abrams & Rowe 1996). For example, most models that predict the evolutionarily optimal age at maturity assume that fecundity increases with body size (e.g. Roff 1984; Stearns & Koella 1986; Berrigan & Koella 1994). Such associations are often found when food availability is held constant (Lyimo and Takken 1993; McCann et al. 2009). However, in our experiment, where food availability varies during the mosquito's development, the environmental factor over two time-periods that determined body size (food availability during early and during late development) affect the life-history traits rather than body size itself. If this is generally the case, it would imply major changes in the way we think about life-history evolution. The timing of resource restriction during development also affected its effect on longevity. We observed only an effect if the restriction was during early development. This is consistent with the common finding that food restriction can slow the ageing process (Weindruch 1996; Shanley and Kirkwood 2000; Mair et al. 2003; Kirkwood and Shanley 2005; Masoro 2005). However, that changing from low to high or from high to low food had negligible effects on longevity contradicts other studies showing that compensatory growth associated with better food conditions reverses the effect of early resource restriction on longevity (Merry 2002; Dhahbi et al. 2004; Spindler 2005). We have no explanation for the difference of these results.

2.4.1 Conclusion

In conclusion, we showed that variability of developmental food conditions in Aedes aegypti mosquitoes has strong effects on adult size, reproductive success and mortality of adult females, with some traits being mostly affected by the food availability in early development and other being affected by late food availability. Such effects may have important consequences for energy allocation strategies, but are generally not considered in model of life-history evolution. We further showed that compensatory growth, which is generally considered an adaptive strategy, does not increase its reproductive success, at least for Aedes aegypti in our laboratory conditions. The reproductive burdens associated with compensatory growth may play an important and limiting role in the evolution of growth and other

related traits. Finally, that the mosquitoes' reproductive success was not directly connected with adult size, but was, rather, influenced by the food conditions that they experienced during development contrasts a central assumption of many ideas in life history theory. Thus, we suggest that our understanding of the evolution of life-histories will be greatly enhanced if we consider the effects of varying the environmental conditions during juvenile development. Such information is important in order to develop effective predictions of disease transmission and strategies of mosquito control.

Chapter 3 Context-dependent relationship between parasite growth and virulence in a microsporidia-mosquito interaction

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Abstract

Many ideas about the evolution of parasites rely on the assumption that their hosts increasingly suffer as parasite load increases, and that the relationship between growth and virulence is similar among environments. We investigated whether the interaction between the parasite *Vavraia culicis* and its host, the mosquito *Aedes aegypti*, is affected by three aspects of the environment: the amount of food available to larvae and to adults, and the age at infection. We measured spore load and estimated the parasite's growth rate and asymptotic load in mosquitoes that died during the experiment and in haphazardly selected, living mosquitoes. In most environments, the probability of infection, spore load, both measures of the parasite's development were higher in dead than in age-matched living mosquitoes, corroborating the idea that virulence increases with the parasite's development. However, these relationships depended on the food availability and age at infection, suggesting that the trade-offs underlying the evolution of virulence depend on the environment. Ideas about the evolution of virulence must therefore consider not only how the environment affects epidemiologically relevant parameters, but also how it affects the relationships between them.

3.1 Introduction

Why do some parasites cause severe pathology and mortality while others are relatively benign? Many theoretical attempts to understand this variation rely on the assumption that there is a trade-off between the rate of transmission and the duration of the transmission period (Anderson and May 1979; Alizon and Lion 2011, Gandon et al. 2001; Dieckmann et al. 2002). Underlying this assumption is the idea that parasites must replicate rapidly to a high load in order to be transmitted efficiently, but that high parasite load (or rapid parasite development) also reduces the host's survival and thus the duration of the transmission period.

Many aspects, however, of the relationship between parasite load and the host's death are obscure. While several examples indeed show that greater parasitemia can increase the risk of death (e.g. HIV (Mellors et al. 1996), rodent malaria (Mackinnon and Read 1999; Mackinnon and Read 2004) and a gregarine parasite of monarch butterflies (De Roode et al. 2008; De Roode and Altizer 2010), many show no relationship (Salvaudon et al. 2007; Little et al. 2008). Indeed, we might expect that the between parasite burden and host mortality is weak for parasites whose symptoms are mainly due to the host's immune response, so that low parasite loads may be as harmful as high loads. For example, clinical malaria and, in particular cerebral malaria, appear to stem in large part from an overactive immune system (Artavanis-Tsakonas et al. 2003).

Another, less explored reason for the variability in the association between parasite development and host mortality is the role of the environment on the association. The environment, in particular resources can affect virulence (Jokela et al. 1999; Brown et al. 2000; Ferguson and Read 2002); resources can also affect parasite growth (Bedhomme et al. 2004; Johnson et al. 2007; de Roode et al. 2008; Hall et al. 2009a). If virulence and growth are affected differently, we might see a negative relationship between parasite development and survival in some environments, but not in others. Indeed, in an experiment with *Daphnia magna* and its intestinal, castrating parasite *Pasteuria ramose*, the relationship between parasite load and mortality rate switched from positive at low food conditions to negative at high ones (Vale et al. 2011). Such studies can be, however, difficult to interpret, as they measure parasite load at the time of the host's death. As parasite load is expected to increase with time after infection, it is intrinsically related to longevity and thus mortality rate. In addition, parasite load in surviving individuals is often not measured, so that we do not know whether dead individuals indeed harbor more parasites than surviving ones. Yet this is a critical assumption if we want to conclude anything about a relationship between parasite load and death. If the environment indeed changes the relationship between the parasite's growth and virulence, testing ideas about the evolution of virulence by changing environmental parameters (as, for example, (Ebert and Mangin 1997; Nidelet et al. 2009))

would be problematic, as the different treatments would differ in the trade-offs underlying the evolutionary predictions.

Here, we study whether the environment (resource availability and age at infection) affects, in addition to host health and parasite development (growth and parasite load), the relationship between the two, using experimental infections of the mosquito, *Aedes aegypti* with the microsporidian parasite *Vavraia culicis*. We compare the number of spores in dead mosquitoes and in a sample of age-matched living mosquitoes. This allows us to decouple the effect of parasite load from the longevity of the host. We further model the development of the parasite within the living and the dead mosquitoes and compare the growth parameters between the two groups. Finally, by manipulate age at infection, larval and adult resources, we find the role of the environment on the relationship between parasite growth and host mortality.

3.2 Material and methods

3.2.1 Experimental system

The microsporidian parasite *Vavraia culicis* was provided by J.J Becnel (USDA, Gainesville, USA). It is an obligate, intracellular parasite that has been reported in natural populations of several genera of mosquitoes including *Aedes* (Weiser and Coluzzi 1972). The host larvae ingest the spores of *Vavraia culicis* with their food, resulting in infection of gut cells and epithelial cells. After several rounds of replication within the larvae the parasite begins to produce its infectious spores. The parasite is transmitted in two ways. First, transmission can occur horizontally from larva to larva, either by spores released in faeces or after larval death. In some cases, e.g. with food stress or heavy infection (Bedhomme et al. 2004), larval death is enhanced. Second, if juveniles survive the infection, they develop into infected adults. The spores are transmitted from females to the next generation of mosquitoes by arriving in a breeding site either when a mosquito dies on a breeding site or on the surface of the eggs (Andreadis 2007). We used the UGAL strain of the mosquito *Ae. aegypti* (obtained from Patrick Guérin, University of Neuchâtel). *Aedes aegypti* occurs throughout the tropics and subtropics, is an important vector for, e.g. dengue and Zika viruses.

3.2.2 Experimental design

The experiment was run in a climate chamber set to 26° C, 70% relative humidity and at 12h light and 12h dark regime. We used a full factorial design, with age at infection (two or five days after hatching),

larval food (the standard diet of our lab (Day 1: 0.06mg of tetramin fish food, day 2: 0.08mg, day 3: 0.16mg, day 4: 0.32mg, day 5: 0.64mg, day 6 or later: 0.32mg) or 40% of the standard diet) and adult food (10% or 2% sucrose-solution) as experimental factors.

Eggs were soaked in deionized water and simultaneously hatched under reduced atmospheric pressure. 2010 first-instar larvae were moved into 12-well plates and kept individually in 3 ml of deionized water. Each larva was haphazardly assigned to one of the eight treatments and fed every 24 hours with the appropriate amount of food. We exposed larvae to infection by adding 2.0 x 10^5 spores in 100µl deionized water per individual. Pupae were moved to 50 ml Falcon tube containing deionized water and a piece of filter paper. The cups were covered with mosquito netting, and mosquitoes were given access to cotton wool moistened with either 10% or 2% sugar solution. To estimate the parasite's growth, we counted spores from all the dead individuals and, starting eleven days after hatching, from a haphazardly selected sample (8-12 individuals) of living mosquitoes of each treatment. The experiment was stopped when all of the mosquitoes had died (32 days after hatching).

3.2.3 Trait measurement

The size of adults was assayed as the mean of their wing length, which strongly correlates with the weight of mosquitoes (Koella and Lyimo 1996) and is widely used as an approximation for adult size. The wings were removed and mounted on microscope slides. The slides were digitally scanned and the wings were measured with the open-access software IMAGEJ. To count the parasite's spores were we placed each mosquito into a 2ml Eppendorf tube containing 180 µl deionized water and a 5mm steal bead. We crushed the mosquitoes by shaking the tube for 4 minutes at 35 Hz (Tissue Lyser, Qiagen, Valancia, California). The steal bead was removed and the spores were counted in a sample of the solution with a haemocytometer (Neubauer improved).

3.2.4 Statistical analysis

We assayed 2014 mosquitoes (between 248 and 253 in each treatment) of which 925 became adult females (between 61 and 131 in each treatment). For the analysis of the juvenile mortality the two adult food treatments were pooled. A generalized linear model (GLM) fitted with a binary logistic regression was performed to determine the effects of larval food, age at infection and their interaction on the likelihood that larvae survived to emerge as adults. We analyzed the proportions of individuals in which we found at least one spore with a GLM (binomial distribution), and the number of spores with a GLM with quasi-Poisson distribution (corrected for overdispersion). For both analyses we included larval food, adult food, age at infection and survival (dead or alive at time of sampling) and their interaction as fixed factors and the time after infection as a nominal confounder. Note that we do not consider

time after infection continuous because of its strongly nonlinear relationship with spore load. Parasite spore loads were $\log_{10}$-transfromed.

We fitted the number of spores using generalized least squares with the following equation

$$n = c * (1 - e^{-k*t})$$

Equation 3:1 - Parasite growth equation

where *n* represents the $\log_{10}$-transformed number of spores, *c* the asymptotic number of spores, *k* a parameter related to the growth rate at low spore loads and *t* the time after infection. We compared *c* and *k* between experimental groups with the gnls function from the nlme package including larval food, adult food, age at infection, survival and all their interactions as fixed factors. The longevity of the mosquitoes was analyzed with a survival analysis that included larval food, adult food, infection time-point and all possible interactions and added wing length as a potential confounder. While we used Weibull distributions because it gave the best fit, Cox proportional hazard gave similar results. All statistical analyses were performed with R version 3.2.3 (R Development Core Team, 2015).

3.3 Results

3.3.1 Juvenile mortality:

The probability that a juvenile died before emergence ranged from 1.8 % (confidence interval of proportion: lower limit = 0.95 %, upper limit = 3.4 %) for mosquitoes that had been infected five days after hatching and reared on high levels of food to 33.2% (CI: lower limit = 29.1 %, upper limit = 37.6 %) for mosquitoes that had been infected at age two and reared on low food. Mosquitoes that had been infected at age five and reared on low food (2.6 %; CI: lower limit = 1.5 %, upper limit = 4.4 %) and at age two and reared on high food (13.5% CI: lower limit = 10.7 %, upper limit = 16.7 %) had intermediate risks of juvenile death. Larval food (χ^2 = 17.9, p < 0.001) and age at infection (χ^2 = 15.9, p < 0.001), but not their interaction (χ^2 = 3.02, p = 0.082), had significant effects influencing juvenile mortality.

3.3.2 Probability of infection, spore load and parasite growth:

The probability of harboring spores significantly increased with infection period and was higher for mosquitoes with a young age at infection (Table 3.1, Figure 3.1A). The analysis of spore load revealed statistically significant effects of time after infection, survival, larval food, age at infection and the interaction between larval food and age at infection (Table 3.1, Figure 3.2B). When mosquitoes died natural-

ly they generally had more spores than living ones at the same age. The spore load of mosquitoes significantly increased with time after infection, and was higher for individuals with high larval food and with a young age at infection.

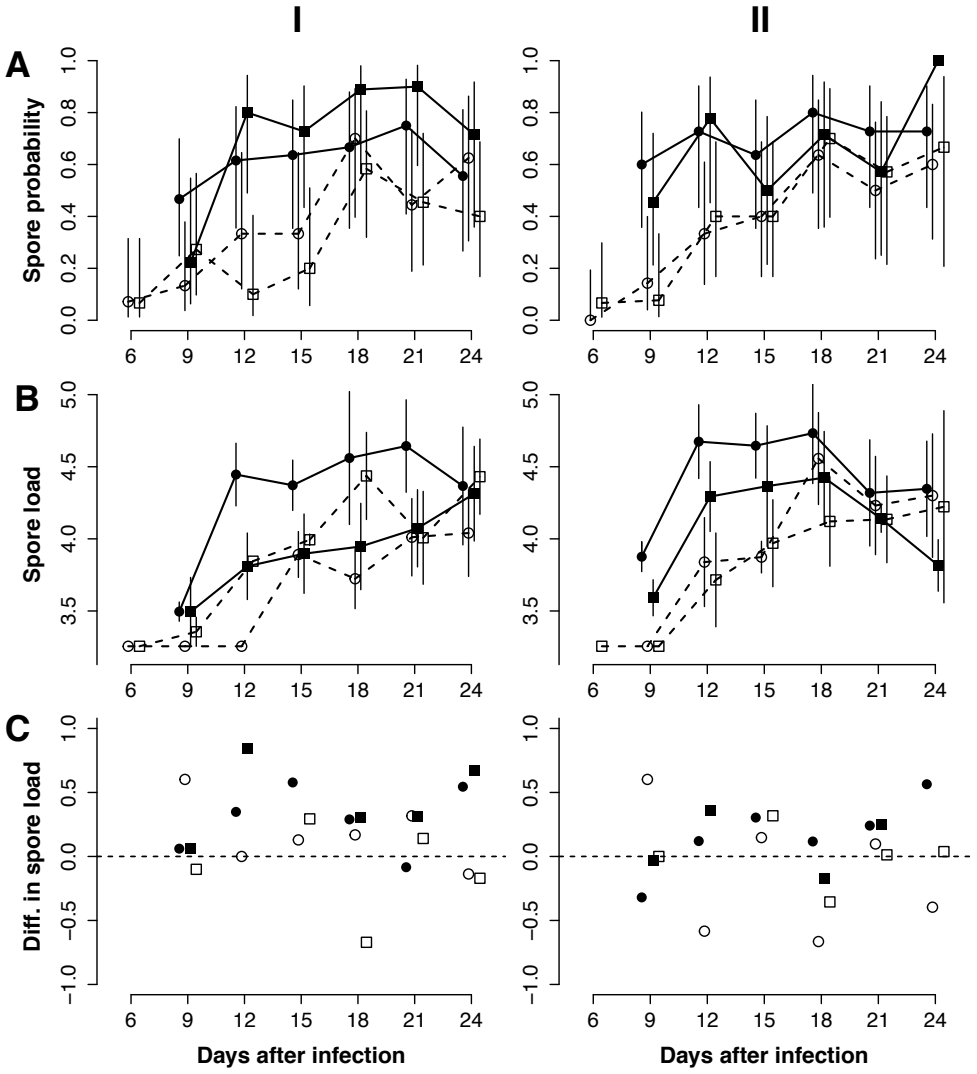

Figure 3:1: Spore load and probability of infection

The effect of larval food availability and age at infection for (A) the probability to find spores (mean ± confidence interval of proportion), for (B) the log number of spores (mean ± se) and for (C) the mean difference of spore load between naturally dead and living mosquitoes. Plotted are, for column I individuals with high adult food availability and for column II individuals with low food adult availability. Solid symbols represent treatments a low age at infection (age two); open symbols represent treatments with an age at infection of five. Circles represent treatments with high food availability during development; squares represent treatments with low food availability during development. The dashed lines in figures C indicate the expectation if spore load would be equal for naturally and artificially dying mosquitoes.

Table 3:1 Spore load and probability of infection

Statistical summary for the probability of infection (GLM with binomial distribution) and spore load (GLM with Poisson distribution corrected for overdispersion). Statistically significant p-values are given in bold.

		Spore probability		Spore load	
Factor	df	χ^2	p	χ^2	p
Survival	1	2.40	0.121	4.89	**0.027**
Larval food	1	1.49	0.223	7.34	**0.006**
Adult food	1	1.39	0.239	1.35	0.245
Age at infection	1	7.51	**0.006**	12.42	**<0.001**
Survival * larval food	1	0.86	0.354	0.23	0.635
Survival * adult food	1	0.58	0.446	1.19	0.276
Larval food * adult food	1	2.44	0.118	0.08	0.779
Survival * age at infection	1	0.73	0.393	1.08	0.298
Larval food * age at infection	1	1.92	0.166	10.17	**0.001**
Adult food * age at infection	1	1.00	0.318	0.61	0.437
Survival * larval food * adult food	1	0.51	0.477	0.29	0.591
Survival * larval food * age at infection	1	0.62	0.431	0.61	0.433
Survival * adult food * age at infection	1	1.32	0.250	0.78	0.378
Larval food * adult food * age at infection	1	3.04	0.081	3.02	0.082
Time after infection	1	54.64	**< 0.001**	60.71	**<0.001**

The growth parameter *k* and the asymptotic spore number c were significantly higher for naturally dying mosquitoes than for living ones (Figure 3.2). While none of the environmental factors significantly influenced *k*, the asymptotic number of spores c was significantly affected by larval food, age at infection, adult food and by some interactions between the factors (summarized in Table 3.2). In particular, the asymptotic spore number was higher for mosquitoes with a low age at infection and for mosquitoes reared on high levels of food as larvae.

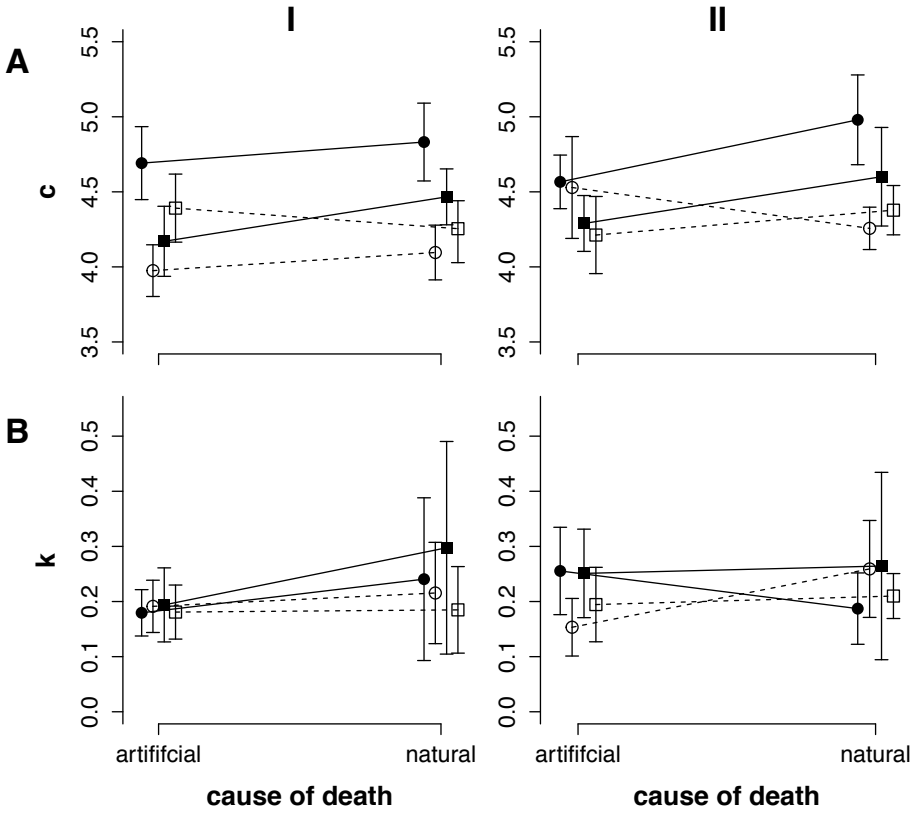

Figure 3:2 Parasite growth parameters

The effect of larval food availability and age at infection for the asymptotic spore load c (A) and the growth parameter *k* (B) ± standard error for (I) individuals with high adult food availability and for (II) individuals with low adult food availability. Solid symbols represent treatments with a low age at infection (age two); open symbols represent treatments with an age at infection of five. Circles represent treatments with high food availability as larvae; squares represent treatments with low larval food availability.

Table 3:2 Parasite growth parameters

Statistical summary for the comparison of *k* (growth parameter) and *c* (asymptotic spore load) between experimental groups. Statistically significant p-values are given in bold.

		Growth parameter		Asymptotic spore load	
Factor	df	F	p	F	p
Survival	1	68.533	**<0.001**	11260.34	**<0.001**
Larval food	1	0.059	0.8086	12.745	**<0.001**
Adult food	1	0.110	0.7398	4.325	**0.0382**
Age at infection	1	0.384	0.5356	16.900	**<0.001**
Survival * larval food	1	<0.01	0.9894	2.136	0.1447
Survival * adult food	1	0.128	0.7203	9.670	**0.002**
Larval food * adult food	1	0.071	0.7904	0.012	0.9115
Survival * age at infection	1	0.251	0.6163	13.574	**<0.001**
Larval food * age at infection	1	0.039	0.8434	26.167	**<0.001**
Adult food * age at infection	1	0.381	0.5376	2.503	0.1143
Survival * larval food * adult food	1	0.063	0.8020	0.053	0.8173
Survival * larval food * age at infection	1	0.450	0.5026	3.499	0.0621
Survival * adult food * age at infection	1	0.086	0.7689	14.462	**<0.001**
Larval food * adult food * age at infection	1	0.805	0.3702	7.070	**0.0081**
Survival * Larval food * adult food * age at infection	1	0.073	0.7870	14.819	**<0.001**

3.3.3 Longevity:

Adult mosquitoes lived longest if they had been infected at age five, reared as larvae on high food and as adults on high food (13.8 days; se = 0.70), and they lived shortest if they had been infected at age 2 and reared on low food as larvae and as adults (3.4 days; se = 0.60). The effects of age at infection, the interaction between larval and adult food, and the three-way interaction between larval food, adult food and age at infection were statistically significant (Table 3.3). Complete data on longevity can be obtained from Figure 3.3.

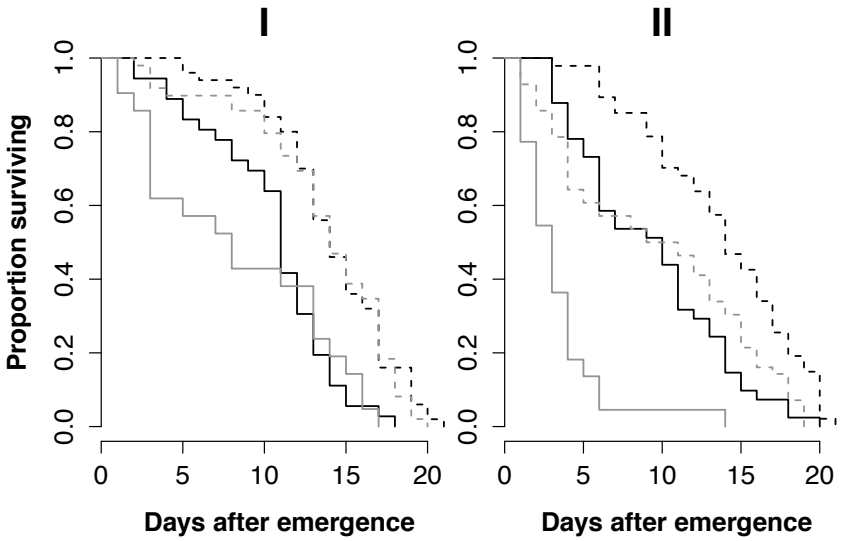

Figure 3:3 Longevity of infected mosquitoes

The effects of larval food and age at infection for longevity of adult females for I) individuals with a low adult food availability and II) for individuals with high adult food availability (age 0 is age after emergence). Dark black lines represent treatments with high food availability as larvae; grey lines represent treatments with low food availability as larvae. Solid lines represent treatments with a low age at infection (age 2); dashed lines represents treatments with high age at infection (age 5).

Table 3:3 Statistical summary of surival analysis

Statistical summary for the survival analysis (Weibull distribution). Statistically significant p-values are given in bold.

	Longevity		
Factor	df	$\chi 2$	p
Larval food	1	0.44	0.509
Adult food	1	0.44	0.509
Age at infection	1	7.49	0.006
Larval food * adult food	1	38.67	< 0.001
Larval food * age at infection	1	0.09	0.769
Adult food * Age at infection	1	0.34	0.561
Larval food * Adult food * age at infection	1	20.98	< 0.001
Wing length	1	0.41	0.522

3.4 Discussion

The food of *Aedes aegypti* and the age at infection greatly altered the dynamics of the host parasite interaction by influencing the probability of infection the growth of the parasite and the longevity of the host. The probability of infection was mainly determined by age at infection, the spore load by lar-

val food and by age at infection and parasite growth was influenced by larval food, adult food and age at infection. The longevity of mosquitoes was mostly determined by age at infection and by interactions between larval food adult food and host age at infection. Furthermore, the probability of infection, the spore load and the spore growth was significantly higher for naturally dying mosquitos than for age-matched living ones, indicating that these traits are important causes of virulence.

In our system, virulence has two components: the probability that infected individuals die before emergence and the longevity of infected adults. Both components were higher if the larvae were younger at infection and if they obtained less food. This is consistent with the literature that less food enhances juvenile mortality (Bedhomme et al. 2004; Lorenz and Koella 2011) and decreases adult lifespan of infected mosquitoes (Lorenz and Koella 2011), and it suggests early infection amplifies these patterns. Although the mechanisms underlying virulence are not known, they may include a direct and density-dependent effect of the parasite (as assumed in many models (Ganusov et al. 2002; Alizon and van Baalen 2005b)) or the depletion of energy below a threshold necessary for the host's survival (Hall et al. 2009b), which is likely to depend not only on parasite load but also on the period of infection.

We were particularly interested in whether the environment affected the way that the parasite's growth and density influenced the severity of parasitism, in particular its host's longevity. Naturally dying mosquitoes generally showed a higher spore load (Figure 3.1C, Table 3.1), a higher growth parameter of the parasite and a higher asymptotic spore load than living ones sampled at the same age (Figure 3.2, Table 3.2). Thus, our data a generally positive relationship between the parasites development and its virulence, in accordance with the central assumption of the virulence transmission trade off (Anderson and May 1982). However we found that the relationship between the host's health and the parasite's development varied between ages at infection and among food levels. Thus the environmental factors influenced the asymptotic spore density (c) differently in living and naturally dying individuals (significant interactions between survival and adult food and between survival and age at infection, Table 3.2).

Age at infection and food availability also interfere in how they express the parasite's exploitation and virulence. Thus, which environmental factors are important differ for the parasite's development and for the host's longevity. Thus, while young age at infection led to higher spore loads and also reduced adult longevity, mosquitoes reared on ample larval food produced more spores (higher growth and parasite load) than mosquitoes reared on little food, but had very similar adult longevity; or the interaction between larval food and adult food availability had little influence on the production of spores, but but a large effect on longevity. It therefore seems that when larval food availability is high the parasite grows with little impact on the host's longevity, but when age at infection is low the host is more vul-

nerable to high parasite loads. The fact that hosts reared on high levels of food seem to be more tolerant to high parasite burden can be interpreted as reduced conflict for shared resources under ample food and is consistent with the findings from Vale et al. (2011) and Zeller and Koella (under review). If we assume that transmission is linked to spore load similarly in all environments (which is, of course not necessarily the case), our results imply that the virulence-transmission trade-off differs among environments, with important consequences for predictions about the evolution of virulence.

Two general mechanisms can explain the effect of resource availability on the outcome of a host-parasite interaction. On the one hand the ample resources can increase the efficacy of the host's immune response and thereby increase the resistance of hosts to parasite infection (Koella and L. 2002; Ayres and Schneider 2009). Malnourished hosts may therefore be weaker and more susceptible to infectious disease (Moret 2000) so that the lower the food availability, the higher the costs of parasitism (Ferguson and Read 2002). On the other hand, the parasites use their host's internal resources for their own development; resources that are normally allocated to the host's growth, maintenance, and reproduction. Accordingly hosts reared on high levels of food may present a better environment for the parasite to develop (Agnew and Koella 1999a; Brown et al. 2000; Bedhomme et al. 2004; Tseng 2006). Because in our study the parasite load was higher for individuals with access to lots of food and when they were infected at a young age, it suggests that the parasite was able to benefit from the host's ample food environment and probably directly accessed the host resources to produces its spores.

3.4.1 Conclusion

We showed that the susceptibility, parasite load and the parasite's within-host growth and virulence are complex, age and resource-dependent traits. Therefore the correlation among ages and food levels between parasite development and virulence can have considerable impact on the evolutionary outcome of infectious disease. The ecological conditions and the age at infection of hosts have therefore the potential to change the relative costs and benefits of parasite replication and are likely to determine the adaptive levels of virulence.

Chapter 4 The role of the environment on the evolution of tolerance and resistance to a pathogen

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Abstract

Defense against parasites can be divided into resistance, which limits parasite burden, and tolerance, which reduces pathogenesis at a given parasite burden. Distinguishing between the two and understanding which defense is favored by evolution in different ecological settings are important, for they lead to fundamentally different evolutionary trajectories of host-parasite interactions. We let the mosquito *Aedes aegypti* evolve under different food levels and either with no parasite, with a constant parasite, or with a coevolving parasite (the microsporidian *Vavraia culicis*). We then tested tolerance and resistance of the evolved lines at the two food levels. Exposure to parasites during evolution increased resistance and tolerance, but there were no differences between the lines evolved with coevolving or constant parasites. Mosquitoes that had evolved with food restriction had higher resistance than those evolved with high food, but similar tolerance. The mosquitoes that had restricted food when being tested had lower tolerance than those with normal food, but there was no difference in resistance. Our results emphasize the complexity and dependence on environmental conditions of the evolution and expression of resistance and tolerance, and help to evaluate some of the predictions about the evolution of host defense against parasites.

4.1 Introduction

Defense mechanisms against parasites can be divided into two broad classes: resistance and tolerance. Resistance reduces the harm caused by disease by preventing infection or limiting the parasite's development within the host, thus leading to lower parasite loads. Tolerance reduces the parasite's detrimental effects without affecting parasite load (Read et al. 2008; Råberg et al. 2009; Little et al. 2010).

While resistance and tolerance both increase the fitness of an infected individual, resistance does so by reducing the parasite's fitness, whereas tolerance does not. Whether hosts evolve tolerance or resistance has fundamentally different effects for the evolutionary trajectory of host pathogen interactions (Roy and Kirchner 2000; Restif and Koella 2003; Boots 2008). Several theoretical studies have predicted how tolerance and resistance might evolve. For example it was predicted (i) that hosts maintain genetic polymorphism for resistance, but not for tolerance (Roy and Kirchner 2000; Miller et al. 2005). The evolution of resistance would provoke counter-adaptation of the parasite to overcome resistance, which would lead to co-evolutionary dynamics with rapidly changing allele-frequencies in resistance-genes. In contrast, the evolution of tolerance would benefit both host and parasite, so enabling its fixation. Thus, (ii) tolerance would increase more easily than resistance (Roy and Kirchner 2000). However, (iii) the parasites could also respond to the evolution of tolerance by increasing their growth to take advantage of the weaker constraint (Miller et al. 2005). Thus, tolerance would only be observed if the hosts are infected with parasites that have not co-evolved with the host. Finally, (iv) parasites with low virulence could be more likely to select for their hosts' tolerance, whereas high virulence could favor resistance (Restif and Koella 2004).

While this short list emphasizes that we expect and can predict different evolutionary outcomes for resistance and for tolerance, these ideas rely on the idea that the two defense strategies are not linked, as for example observed in natural populations of monarch butterflies and (Lefèvre et al. 2010) and a cyprinid fish (Mazé-Guilmo et al. 2014) and their parasites. In other systems, however, evolution of the two defense strategies appears to be constrained by a negative genetic correlation. For example, mice infected with *Plasmodium chabaudi* show a negative genetic correlation between resistance (peak pathogen load) and tolerance (weight and red blood cells) (Råberg et al. 2007). This could be due to linkage disequilibrium, pleiotropic effects or physiological constraints.

Further complicating the evolutionary pressures is the fact that the environment, and in particular resource availability can affect the predictions mentioned above. First, because resource availability can influence not only the host's ability to tolerate parasites, but also how the host's genotype affects its (Sternberg et al. 2012; Howick and Lazzaro 2014; Mazé-Guilmo et al. 2014), tolerance is likely to evolve

differently under different food environments. Second, because food availability often alter virulent parasitic effects (including the *Ae. aegypti* - *V.culicis* system that we study (Bedhomme et al. 2004; Lorenz and Koella 2011)), it should influence the evolution of tolerance and resistance (Restif and Koella 2004). Third, the evolutionary cost of resistance depends on the availability of resources, so that different environments constrain evolution of the two traits in different ways (Hochberg and Baalen 1998; D. C. Lopez-Pascua and Buckling 2008; Boots 2011; Harrison et al. 2013).

We studied such aspects of the evolution of resistance and tolerance with the mosquito *Aedes aegypti* and its microsporidian parasite *Vavraia culicis*. With an experimental evolution approach we let the hosts evolve in response to parasites in different environments. Our general goal was to evaluate some of the ideas mentioned above. We therefore investigated (i) whether our system has enough genetic variability and low enough costs to enable the evolution of resistance and tolerance, (ii) how resource availability influences the evolution of tolerance and resistance, and (iii) whether coevolving parasites influence the two defense traits differently than constant parasites.

4.2 Material and methods

Our experiment was run in a climate chamber set to 26° C, 70% relative humidity and a 12h light and 12h dark regime.

4.2.1 Experimental system

We used the microsporidian parasite *Vavraia culicis* (provided by J. Becnel, USDA Gainesville) and the UGAL strain of one its mosquito hosts, *Ae. aegypti* (provided by P, Guérin, University of Neuchâtel). *Aedes aegypti* occurs throughout the tropics and subtropics, is an important vector for, e.g. dengue and Zika viruses.

Vavraia culicis is an obligate, intracellular parasite that infects several genera of mosquitoes, including *Aedes* (Weiser and Coluzzi 1972). Mosquito larvae ingest the parasite's spores with their food, resulting in the infection of gut and epithelial cells. After a period of replication within the larva the parasites begin to produce their infectious spores, which are transmitted in two ways. First, transmission can occur from larva to larva when spores are released after the larva dies. This transmission route is enhanced by food stress or strong infection (Bedhomme et al. 2004). Second, if larvae survive the infection to develop into adults the spores can be released when the mosquito dies in the aquatic environment or they can adhere to the surface of the eggs and infect the newly hatched larvae (Andreadis 2007).

4.2.2 Experimental evolution

We let the mosquito evolve for 10 generations (i) either without the parasite, with an externally maintained parasite, or with a co-evolving parasite, and (ii) either with high or with low food availability during the larval stage. The 'constant' parasites were taken from our standard line maintained in our mosquito colony. The co-evolving parasites were taken from the previous generation of the infected mosquitoes. Each treatment was replicated three times. The first generation of the experiment was created by haphazardly moving 200 one-day old larvae from the colony to each line. To rear the mosquitos, we hatched eggs simultaneously under reduced atmospheric pressure. For the first four days we reared for each line 50 larvae in 4 petri dishes (8 cm diameter) containing 30 ml deionized water (Rearing the larvae in petri dishes rather than a large tray helps to obtain a successful infection). Every 24 hours we fed the larvae with our standard amount of food (Day 1: 0.06mg of tetramin fish food, day 2: 0.08mg, day 3: 0.16mg, day4: 0.32mg, day 5: 0.64mg, day 6 or later: 0.32mg) or with half of the standard diet. We exposed the larvae to infection two days after hatching by adding 5.0 x 10^6 *Vavraia culicis* spores in 1 ml deionized water. In the first generation constant parasite and coevolution treatments received the same solution of spores prepared from the standard lab colony. Two days after infection the four groups of larvae from each line were moved to a 200 * 150 * 50 mm plastic tray containing 1.5 l deionized water. Pupae were transferred into cages (30x30x30 cm size) containing filter paper soaked with 10% sugar solution as food and a cup containing deionized water and a piece of filter as an oviposition substrate. Four, six, eleven and thirteen days after the day when 75% of mosquitoes of a given line pupate they were given the opportunity to take a bloodmeal on MZ's arm for 8 minutes. The eggs were removed every 48 hours and stored at 26° C and 70% relative humidity until the start of a new generation. For the co-evolved parasite population (coevolution) we collected the dead infected mosquitoes (larvae and adults), ground them in an eppendorf tube, counted the spores and kept them at 5°C until the next generation of hosts was started. Before starting a new generation we eliminated *Vavraia* spores from the eggs by bleaching the eggs of all lines with 1% household bleach.

4.2.3 Measuring result of experimental evolution

After 10 generation of evolution we measured spore load and longevity of the mosquitoes from all evolved lines exposed to *V. culicis* spores from the lab colony and fed either with the standard amount of food or with half of the standard diet. We reared the mosquitoes as described above, with the following differences. First, we reared the larvae individually in 3ml deionized water in the wells of 12-well plates. We had between 109 and 112 first-instar larvae per line (in total 2009 larvae). Each larva was haphazardly assigned to the high or the low food treatment (between 54 and 56 individuals per treatment and line). Second, we exposed larvae to the parasite by adding 100µl of a solution containing 2.0 x

10^6 *Vavraia culicis* spores per ml deionized water. Third, pupae were moved to 50ml Falcon tube containing deionized water and a piece of filter paper. The cups were covered with mosquito netting, and cotton wool moistened with 10% sugar solution was placed onto the netting remoistened every 48 hours and changed every 72 hours. One day after emergence the males were discarded and the females where checked every day for survival. The experiment was stopped when all of the mosquitoes had died (57 days after hatching).

4.2.4 Spore measurement

Vavraia culicis spores were measured with a haemocytometer. Each mosquito was individually placed into a 2ml Eppendorf tube containing 200µl distilled water and a 5mm steal bead. Mosquitoes were crushed by shaking the tube for 3 minutes at 35 Hz (Tissue Lyser, Qiagen, Valancia, California). Eight µl of the mix were added to the haemocytometer (Neubauer improved) and the spores were counted with a cell counter.

4.2.5 Measurement of host resistance and tolerance

We measured two types of resistance: qualitative resistance as the proportion of individuals in which we found spores, and quantitative resistance as the inverse of the spore load. We measured tolerance as the slope between longevity and spore load at the time of death (Råberg et al. 2009). Note that all mosquitoes died later than 14 days after infection (~ seven days after emergence), at which time the spore load has generally reached an asymptotic value (Zeller and Koella, in prep.). Therefore, it is expected that spore load would not further increase with the mosquito's age and thus that it is not autocorrelated with age at death.

4.2.6 Statistical analysis

All analyses were done with R v. 3.2.3 (R Development Core Team, 2015). Differences in the probabilities of infection were analyzed with a generalized linear mixed effect model (GLMM; binomial errors, logit link, using the lme4 package) that included food level, the two factors (parasite and food level) during the evolutionary history and all their interaction as fixed factors, and replicate of the evolution treatment as a random effect nested within evolution treatment. Spore load of infected individuals was analyzed with a linear mixed effect model that included the same factors. Longevity of adult mosquitoes was analyzed with a linear mixed effect model that included food, parasite during evolution and food during evolution as fixed factors, spore load as continuous variable and replicate nested within the evolution treatments. A significant interaction between spore load and experimental factors indicate differences in tolerance (Simms 2000; Råberg et al. 2007). The number of spores (+ 1) was $\log_{10}$-

transfromed for all analyses. Full models included all possible interactions. Minimal models were derived by removing unsignificant terms followed by model comparisons with likelihood-ratio tests. If removing a term significantly reduced the explanatory power of the model, it was kept in the model.

The relationship between resistance (mean inverse parasite burden) and tolerance (slope between parasite burden and longevity) was analyzed with linear regression that considered food availability and the interaction between food availability and tolerance as factors.

4.3 Results

A total of 1814 out of 2009 (90%) mosquitoes survived to adulthood. 881 (48.6%) of these were females and were analyzed (between 18 and 33 individuals per line and treatment).

Qualitative resistance was affected by none of our experimental factors (Table 4.1). Quantitative resistance was affected by parasitism and food level during evolution (Table 4.1, Figure 4.1). When mosquitoes were exposed to parasites during evolution they generally showed higher resistances. Post-hoc test between coevolution and constant parasite treatments showed no significant differences for resistance (analysis not shown). Mosquitoes originating from lines with food restriction during evolution had a higher resistance. Neither food availability nor interactions among factors had an effect for resistance.

Table 4:1 Statistical summary for resistance

Statistical summary for quantitative (spore load) and qualitative (probability of infection) resistance analysis.

	Spore Load			Probability of infection	
Factor	df	χ^2	p	χ^2	p
Food	1	0.19	0.667	>0.01	0.953
Evolution parasite	2	6.05	**0.049**	2.89	0.236
Evolution food	1	7.13	**0.008**	0.52	0.473
Food * Evolution parasite	2	1.66	0.436	1.91	0.384
Food * Evolution food	1	1.19	0.276	0.09	0.767
Evolution parasite * Evolution food	2	1.84	0.398	0.40	0.819
Food * Evolution parasite * Evolution food	2	1.40	0.497	1.96	0.375

Tolerance significantly varied between food treatments and between lines with different types of parasitism during evolution (significant interaction between spore load and food, and between spore load

and evolution parasite (Table 4.2, Figure 4.1)). Tolerance was significantly higher for lines with high food availability and for lines originating from lines that were exposed to parasites during evolution. Post-hoc test between coevolution and constant parasite treatments showed no significant differences (analysis not shown). We found no difference in tolerance between lines reared at the two levels of food during evolution. The longevity of infected mosquitoes (when controlled for parasite induced fitness loss) was significantly influenced by the interaction between evolution parasite and evolution food (Table 4.2). The longevity was generally higher for lines that had evolved with parasites and higher food levels. The interaction between food and evolution parasite had a close to significant effect on the longevity.

Table 4:2 Statistically summary for tolerance

Statistical summary for longevity analyses. Significant interactions between parasite load and experimental factors indicate differences in tolerance.

		Longevity	
Factor	df	Chisq	p
Parasite load	1	2.53	0.111
Food	1	1.19	0.275
Evolution parasite	2	0.70	0.706
Evolution food	1	2.50	0.114
Parasite load * Food	1	18.95	**< 0.001**
Parasite load * Evolution parasite	2	7.03	**0.030**
Food * Evolution parasite	2	4.89	0.087
Evolution parasite * Evolution food	2	8.50	**0.014**

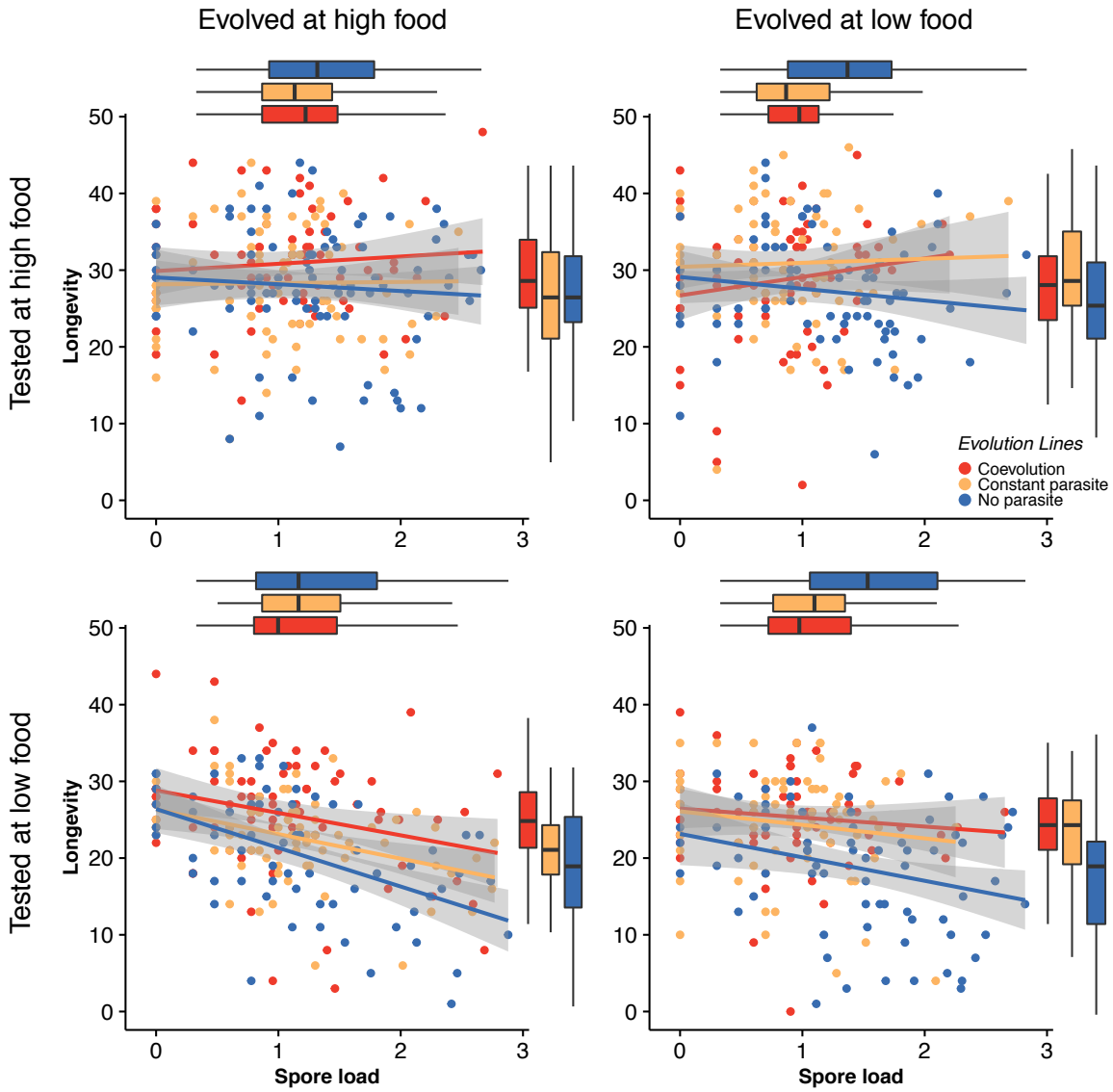

Figure 4:1 Tolerance and resistance

Aedes aegypti tolerance and resistance to Vavraia culicis for mosquitoes from different evolutionary lines and tested at different food levels. Box-plots above and to the right each panel show the median, the 25th and 75th quantile and the range of longevity and spore load. Red dots and box-plots represent individuals originating from lines with coevolving parasites, yellow dots represent individuals with constant parasites during evolution and blue dots represent individuals that had evolved without parasites. Lines show least-squares regressions for different evolution lines. Panels in the first row represent mosquitoes tested at high food; those in the second row represent mosquitoes tested at low food. Panels in the first column represent lines that had evolved at high food availability; those in the second column represent lines that had evolved at low food availability. Individuals from different replicates are pooled.

We found a significant positive correlation between resistance and tolerance (f = 4.60 p = 0.040). Food availability (f = 0.07 p = 0.795) and the interaction between food availability and tolerance (f = 1.93, p = 0.174) had non-significant effects in determining resistance (Figure 4.2).

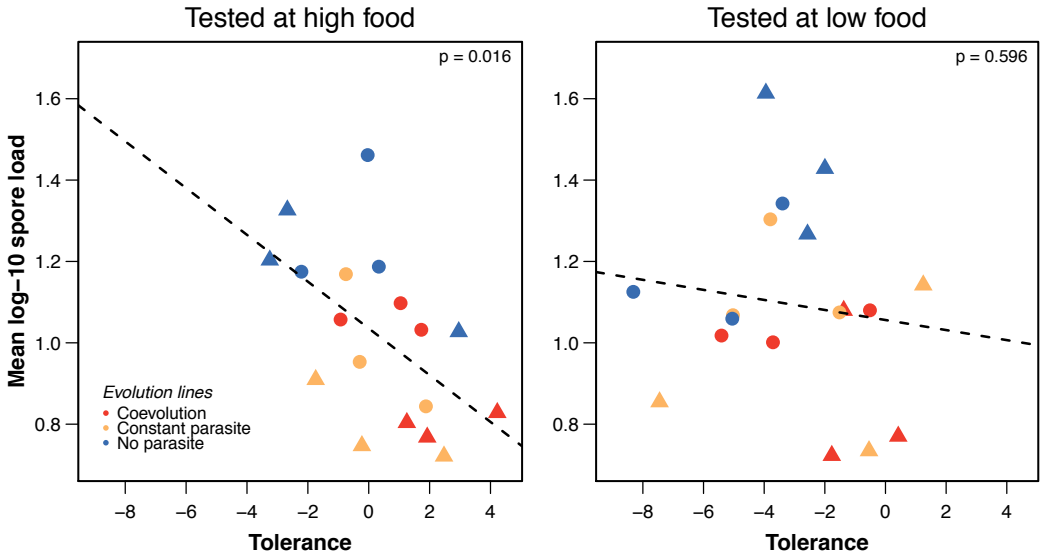

Figure 4:2 Tolerance vs. resistance

The relationship between spore load and tolerance for mosquito lines with different evolutionary origin. Note that the resistance increases from top to bottom, so a negative slope implies a positive association between resistance and tolerance. Dots represent mean spore load and tolerance of mosquitoes that had evolved at high food availability; triangles represent mosquitoes that had evolved at low food availability. Red symbols represent individuals that had evolved with coevolving parasites, yellow symbols represent individuals that had evolved with constant parasites and blue symbols represent individuals that had evolved without parasites. The panel on the left shows mosquitoes tested at high food; the one on the right shows mosquitoes tested at low food. Lines show least-squares regressions pooled for all lines. The p-values on this figure were calculated for each food treatment separately

4.4 Discussion

In our colony of *Ae. aegypti* mosquitoes, tolerance of and resistance to the microsporidian parasite *V. culicis* are evolvable traits, so that mosquito lines that were exposed to parasitism during evolution show both a higher resistance and tolerance to parasitism. However the way these two defense traits evolve depends on the ecological settings. Thus, restricting food during evolution led to higher resistance, but had no impact on tolerance. Furthermore, the ecological settings during the testing of the

mosquitoes also affected the observed defense strategies. Thus, food restriction generally decreased tolerance, but had no effect on resistance.

The mosquitoes' parasite load and the reduction of longevity with increasing parasite burden was lower for lines that had exposed to parasites during evolution, and indicates a genetic variation in resistance and tolerance for those lines. This is inconsistent with models that predict evolutionary fixation of tolerance alleles (Roy and Kirchner 2000; Miller et al. 2006). At least five other studies found evidence for variation in tolerance (Råberg et al. 2007; Vale and Little 2009; Howick and Lazzaro 2014; Regoes et al. 2014; Lough et al. 2015); two not (Lefèvre et al. 2010; Hayward et al. 2014).

That tolerance and resistance both increased as an evolutionary response to microsporidia infection suggests that there is no major internal constrain in the form of a string negative genetic correlation between the two traits. The positive association between the two traits observed after evolution could be due to independent evolution of the two traits or due to a positive genetic correlation between the two traits. We have no indication from our study to suggest which of the two possibilities is correct. The possibility of a positive correlation is plausible, and such a correlation was found in at least one other study (Howick and Lazzaro 2014), although most other studies show either no genetic correlation (Sternberg et al. 2012; Mazé-Guilmo et al. 2014) or a negative one (Råberg et al. 2007; Vincent and Sharp 2014). However, the fact that different evolutionary environments led to different evolutionary pathways suggests that any possible positive genetic correlation was not strong enough to constrain the evolution of the two defense strategies, so that the two can evolve more or less independently. The observed positive correlation also emphasizes that correlations among population should not be considered as evidence for trade-offs or positive links, as they result from a combination of selection on both traits and the genetic correlation between them (Simms and Triplett 1994; Restif and Koella 2004).

We found no differences in resistance and tolerance between lines that were exposed to coevolving parasites or constant parasites. Accordingly our data does not support both of the self-contradictory predictions that coevolution either increases tolerance because of its lower impact on the parasites fitness (Roy and Kirchner 2000) or decreases because parasites would respond to tolerant hosts by growing faster (Miller et al. 2006). However, the duration of the experiment might have been too short to differentiate tolerance between lines with coevolving or constant parasites.

Even though food had no direct influence on resistance, mosquitoes evolved resistance more easily in resource-poor environments, contrasting findings that high food levels tend to result in the evolution of elevated resistance (Hochberg and Baalen 1998; D. C. Lopez-Pascua and Buckling 2008; Boots 2011; Harrison et al. 2013), probably because resistance is less costly when there resources are plentiful.

However, because in our system well-fed mosquitoes evolved to be tolerant to infection, mosquitoes might have benefited only slightly from resistance. In resource-poor environments, where the fitness loss with increasing parasite burden was much higher, resistant individuals might have been under positive selection and increased with time in frequency. In other words, the evolutionary trajectory of resistance in different environments could depend on how the environment influences tolerance. These results are in accordance with the prediction that higher levels of virulence (in our case triggered by resource restriction) results in the evolution of increased resistance (Restif and Koella 2004). An alternative explanation is that the cost of resistance is only apparent in good environments. Nevertheless the fact that resistance can more easily evolve in resource-poor environment is surprising because resource-harsh environments can therefore increase the host ability to deal with parasites.

A considerable part of the mosquito's longevity is explained by factors that are not related with parasite burden. Mosquitoes that were exposed to parasites during evolution generally lived longer. This can be explained by potential costs of quantitative resistance or by evolving a longer uninfected longevity. In uninfected mosquitoes this trend was similar (Zeller & Koella in prep.) suggesting that the conditions during evolution affected the evolved longevity (general vigor).

4.4.1 Conclusions

Our study is the first experimental test, which found enough genetic variability and low cost that tolerance and resistance evolved when faced to parasites and under different resource levels. The fact that different combinations of resistance and tolerance evolved in different ecological settings illustrates the importance to study those traits across environmental variables. In addition because many of the predictions did not hold true in our system underlines the importance to incorporate such environmental heterogeneity, condition-dependent evolutionary costs and non-independence between both traits when modeling the evolution of resistance and tolerance. Our study provides further an example of how resources and the ability to tolerate parasites might interact to determine the evolution of resistance.

Chapter 5 Antagonistic coevolution and resources alter the host's life history evolution

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Abstract

Early reproduction as an adaptive consequence of parasitism has been predicted by life history theory. However, empirical validation of this prediction is limited and direct experimental evidence that parasites can influence the host's genetically determined development time is missing. We have set up an evolution experiment by letting *Aedes aegypti* mosquito evolve either with no parasite, with a constant parasite, or with a co-evolving parasite (*Vavraia culicis*) with either high or low resource availability. We tested the host's life history traits of the evolved lines at high and at low food availability with or without parasites. We found that when mosquitoes were exposed to parasites during evolution they had a shorter genetically determined development time, an equal body size and a longer longevity compered to mosquitoes originating from control lines. These results suggest no evident trade-offs among age at maturity and other traits. We also found no differences in age at maturity between lines with co-evolving or constant parasites, but lines with co-evolving parasites lived significantly longer. The environmental conditions influenced the host's life-histories and the phenotypic plastic responses to parasitism in many ways, suggesting that variable environments influence the long-term host evolution.

5.1 Introduction

The co-evolutionary interactions between hosts and parasites are recognized as important factors shaping hosts life history evolution (Hochberg et al. 1992; Koella et al. 1998; Koella and Restif 2001; Gandon et al. 2002; Ashby and Boots 2015). Parasites modify the ecological context in which host traits evolve by affecting components of the host's age-dependent fecundity and mortality. This selection pressure can influence the host's optimal pattern of resource-allocation.

There is increasing evidence that hosts may alter their life history traits in a way to compensate for the negative effects of parasitism (Minchella 1985; Koella et al. 1998; Richner 1998; Agnew et al. 2000). Life history theory predicts that earlier reproducing hosts will have a selective advantage, because they might be able to evade parasitism in time and, when parasitized, reduce the impact of parasitism on reproductive success and survival (Hochberg et al. 1992; Forbes 1993; Perrin and Christe 1996). Life history traits of the host, which have been shown to respond to parasitism, include early versus late fecundity (Minchella and Loverde 1981; Gérard and Théron 1997; Adamo 1999), reproductive effort (Sorci et al. 1996; Polak and Starmer 1998; Krist 2001), parental care (Christe et al. 1996; Richner and Tripet 1999), body size (Lafferty 1993; Pontier et al. 1998; Arnott et al. 2000) and developmental time (Agnew et al. 1999; Jones et al. 2008). In some cases these modifications had a genetic underpinning (Lafferty 1993; Koella and Agnew 1999), in others, they were plastic responses. However, an optimization in one trait (e.g. maturing early) might be associated with penalties in another trait, such as maintenance functions, immune-related traits or late-life reproduction. This can be induced by resource-allocation trade-offs, linkage disequilibrium or pleiotropy.

Such modification in life history can be seen as a form of resistance, which might lead to complex co-evolutionary dynamics between the life histories of the host and the parasites. Because life histories of hosts and parasites are at least partly determined by the genotype of the counterpart (Koella and Agnew 1999), an evolutionary response of host's life history to parasitism may again alter the selection pressure of the parasite. This reciprocal selection might result in coevolution, with continuous changes of hosts and parasites life-histories (Gandon et al. 2008; Gaba and Ebert 2009). According to the red-queen hypothesis the evolutionary rates of change in genes related to resistance traits should be accelerated through co-evolutionary dynamics. Indeed, genomes of coevolving hosts have shown to evolve faster compared to populations evolving against a constant parasite population (Paterson et al. 2010; Kashiwagi and Yomo 2011). Theoretical studies reveal that co-evolutionary dynamics can have many effects for the evolution of the host life history (Koella and Restif 2001; Restif et al. 2001; Gandon et al. 2002; Ashby and Boots 2015), however direct experimental evidence that parasites, and in particular, co-evolving parasites can influence the host's genetically determined life history is missing.

Contrarily to the evolved adaptations in life history traits, many hosts respond to parasitism and food restriction phenotypically plastic (including *Ae.aegypti* - *V.culicis* system that we study). A lot of hosts decrease their growth, mature later and with a smaller body size once infected (Bedhomme et al. 2004). In addition to the life history traits themselves, the degree of phenotypic plasticity, the ability of single genotypes to produce more than one phenotype across different environments (Pigliucci 2001), is also genetically determined. If there exists adaptive variation for phenotypic plasticity among genotypes, it is likely to evolve differently under variable conditions. However, how phenotypic plasticity evolves experimentally has rarely been investigated.

We use an experimental evolution approach with the mosquito *Aedes aegypti* and its microsporidian parasite *Vavraia culicis* to examine how the life histories of the host change, by letting hosts evolve in response to parasites under different resource levels. Studying different levels of resources can be relevant because potential trade-offs between different life history traits might only be detectable when resources are scarce. Specifically we want to investigate: (i) whether an exposition to parasites over several generation leads to early maturing host's, (ii) whether evolved early maturity is associated with costs later in life, (iii) whether co-evolving parasites influence the hosts life history evolution differently than constant parasites (iv) and whether our mosquito colony has enough genetic variability and adaptive differences to enable the evolution of phenotypic plasticity.

5.2 Materials and methods

The experiment was run in a climate chamber set to 26° C, 70% relative humidity and a 12h light and 12h dark regime.

5.2.1 Experimental system

We used the microsporidian parasite *Vavraia culicis* (provided by J. Becnel, USDA Gainesville) and the UGAL strainone of its mosquito hosts, *Aedes aegypti* (provided by P, Guérin, University of Neuchâtel).

Vavraia culicis is an obligate, intracellular parasite that infects in nature several genera of mosquitoes, including *Aedes* (Weiser and Coluzzi 1972). The mosquito larvae ingest the spores of *Vavraia culicis* with their food, resulting in infection of gut cells and epithelial cells. After a period of replication within the larvae the parasites begin to produce their infectious spores, which are transmitted in two ways. First, transmission can occur from larva to larva when spores are released after the larva dies. This transmission route is enhanced by food stress or strong infection (Bedhomme et al. 2004). Second, if larvae survive the infection to develop into adults the spores can be released when the mosquito dies in the

aquatic environment or they can adhere to the surface of the eggs and infect the newly hatched larvae (Andreadis 2007).

5.2.2 Experimental evolution

We let the mosquito evolve for 10 generations (i) either with no parasite, with an externally maintained parasite, or with a co-evolving parasite, and (ii) either with high or with low food availability during the larval stage. Each parasite treatment evolved. The 'constant' parasites were taken from our standard line maintained in our mosquito colony. The co-evolving parasites were taken from the previous generation of the infected mosquitoes. Each treatment was replicated three times. The first generation of the experiment was created by haphazardly moving 200 one-day old larvae from the colony to each line.

To rear the mosquitos, we hatched eggs simultaneously under reduced atmospheric pressure. For the first four days we reared for each line 50 larvae in 4 petri dishes (8 cm diameter) containing 30 ml of deionized water in order to ensure a successful infection. Every 24 hours we fed the larvae either with our standard amount of food (Day 1: 0.06mg of tetramin fish food, day 2: 0.08mg, day 3: 0.16mg, day4: 0.32mg, day 5: 0.64mg, day 6 or later: 0.32mg) or with half of the standard diet. We exposed the larvae to infection two days after hatching by adding 5.0×10^6 *Vavraia culicis* spores in 1 ml deionized water. In the first generation constant parasite and coevolution treatments received the same solution of spores prepared from the standard lab colony. Two days after infection the four groups of larvae from each line were moved to one 200 * 150 * 50 mm plastic tray containing 1.5 liter of deionized water. Pupae were transferred into cages (30x30x30 cm size) containing sugar solution and a cup containing deionized water and a piece of filter as an oviposition substrate. Four, six, eleven and thirteen days after the day when 75% of mosquitoes of a given line pupated they were given the opportunity to take a bloodmeal on MZ's arm for 8 minutes. The eggs were removed every 48 hours and stored at 26° C and 70% relative humidity until the start of a new generation. For the co-evolved parasite population (co-evolution) we collected the dead infected mosquitoes (larvae and adults), ground them in an eppendorf tube, counted the spores and kept them at 5°C until the next generation of hosts was started. Before starting a new generation we eliminated *Vavraia* spores from the eggs by bleaching the eggs of all lines with 1% household bleach.

5.2.3 Measuring result of experimental evolution

After 10 generation of evolution we measured the probability of emergence, age at pupation, adult size and longevity of the mosquitoes from the evolved lines. Mosquitoes from the 18 selection lines were exposed to *V.culicis* spores from the lab colony and fed either with the standard amount of food or with half of the standard diet. We reared the mosquitoes as described above, with the following differences.

First, we reared the larvae individually in 3ml deionized water in the wells of 12-well plates. We had between 219 and 224 first-instar larvae per line (in total 4005 larvae). Each larva was haphazardly assigned to one of the four treatments (between 53 and 56 individuals per treatment and line). Second, we exposed larvae to the parasite by adding 100μl of a solution containing 2.0 x 10^6 *Vavraia culicis* spores per ml deionized water. Third, pupae were moved to 50ml Falcon tube containing deionized water and a piece of filter paper. The cups were covered with mosquito netting, and cotton wool moistened with 10% sugar solution was placed onto the netting remoistened every 48 hours and changed every 72 hours. One day after emergence the males were discarded and the females where checked every day for survival. The experiment was stopped 69 days after hatch when all of the mosquitoes had died.

5.2.4 Trait measurement

The size of adults was assayed as the mean of their wing length, which strongly correlates with the weight of mosquitoes (Koella and Lyimo 1996) and is commonly used as an approximation for adult size. The wings were removed and mounted on microscope slides. The slides were digitally scanned and the wings were measured with the open-access software IMAGEJ.

5.2.5 Statistical analysis

Differences in the probabilities of emergence were analyzed with generalized linear mixed effect model (binomial distribution) that included parasite infection, food level, the two factors (parasite and food level) during the evolutionary history and all their interaction as fixed factors. Replicate was treated as random effect, nested within evolution treatment. We used the glmer function from the lme4 package from R v. 3.2.3 (R Development Core Team, 2015). Age at pupation and longevity were analyzed with a mixed effect survival analysis (cox-proportional hazard) that included parasite infection, food availability, parasite during evolution and food during evolution as fixed factors, replicate was treated as a random effect, nested within the evolution treatments. In the analysis of longevity, we added wing length as a potential confounder. Significant interactions between evolutionary factors and tested factors indicate evolutionary differences in phenotypic plasticity. Wing length was analyzed with a linear mixed effect model (lmer function from lme4 package) that included parasite infection, food availability, parasite during evolution and food during evolution and all their interaction as fixed factors, replicate as random effect, nested within evolution treatment.

5.3 Results

A total of 3716 out of 4005 (93%) mosquitoes survived to adulthood. 1764 (47.5%) of these were females and were analyzed (between 17 and 33 individuals per line and treatment). Food variability, parasite infection and their interaction strongly influenced the mosquitos' life history (Table 5.1, Figure 5.1, 5.2 & 5.3). We will focus here on how the food availability and the type of parasitism during evolution influenced the mosquito's life history and how these factors influenced the mosquito's phenotypic plastic response to food stress and parasite infection. Because many factors and interactions influenced the mosquitos' life history we describe only the results, which seem most important to us.

5.3.1 Age and size at maturity

The age at pupation was significantly influenced by the type of parasitism during evolution (Table 5.1, Figure 5.1)). When mosquitoes were exposed to parasites during evolution they matured at a lower age. This was particularly the case when infected with *V.culicis* (significant interaction between parasite infection and type of parasitism during evolution). Tukey's HSD *post hoc* test between coevolution and constant parasite treatments showed no significant differences for age at pupation (analysis not shown). In addition the type of parasitism and food availability during evolution affected the phenotypic plastic response to parasitism and food restriction (significant interactions between evolution treatments and "tested" treatments). For example, mosquitoes that were not exposed to parasites and raised on ample food during evolution (control high food), showed in developmental time the highest phenotypic plastic response when exposed to parasitism (for high and low food (Figure 5.2a and 5.2c).) Contrary to that, we find the lowest phenotypic plastic response to food restriction for individuals, which were not exposed to parasitism and had low food availability during evolution (control low food, Figure 5.2b).

The wing length was significantly influenced by food availability and by the interaction between food and parasite infection. It was lowest for parasitized mosquitoes with low food availability. Furthermore the three-way interaction between parasite infection, type of parasitism and food availability during evolution as well as the three-way interaction between food availability, type of parasitism and food availability during evolution had significant effects in determining the wing length (Figure 5.1 and Table 5.1). The phenotypic plastic response to food restriction and to food restriction combined with parasite infection was smallest for coevolved mosquitoes with high larval food availability (Fig. 5.2 B & C). Tukey's HSD *post hoc* test between coevolution and constant parasite treatments showed no significant differences (analysis not shown).

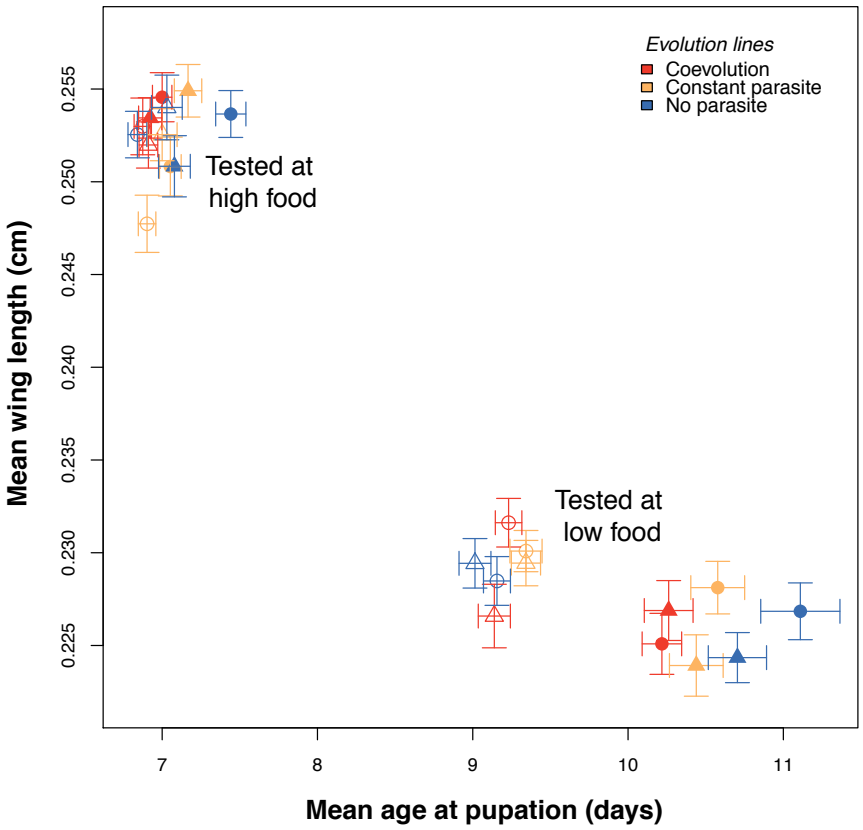

Figure 5:1 Age and size at maturity

Mean wing length ± SE against mean age at pupation ± SE. Red symbols represent coevolution lines, yellow symbols represent lines with constant parasites, and blue symbols represents lines without parasites. Triangles represent lines that had low food availability during evolution; squares represent treatments with high food availability during evolution. Open symbols represent treatments that were not infected; filled symbols represent treatments that were infected with parasites. Individuals from different replicates are pooled.

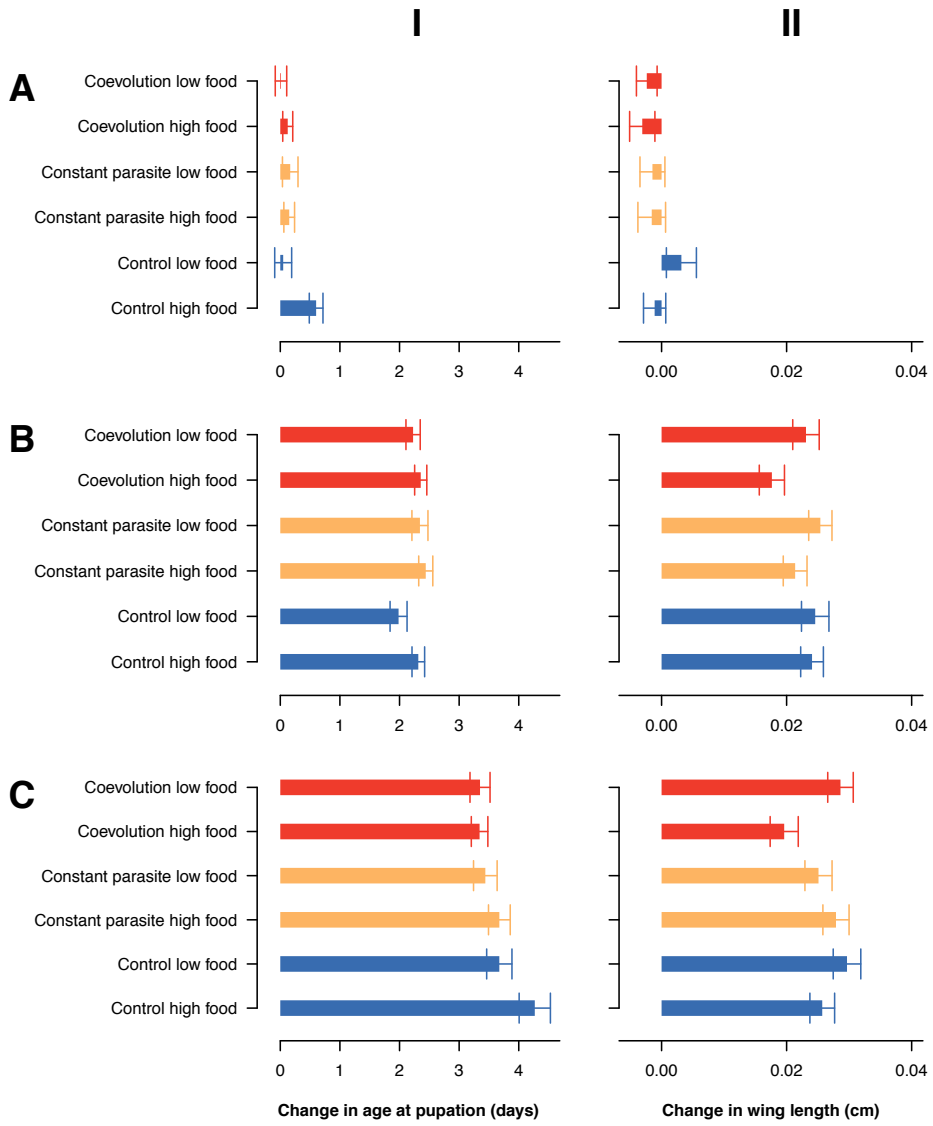

Figure 5:2 Phenotypic plasticity

Phenotypic plastic response to A) parasite infection, B) food restriction and C) parasite infection and food restriction for column I) age at pupation and column II) wing length. Phenotypic plasticity was estimated for each evolution treatment. We calculated the distance between the mean values ± SE from high food un-parasitized treatments to high food parasitized, low food un-parasitized and low food parasitized treatments. Individuals from different replicates are pooled.

5.3.2 Probability of emergence and adult longevity

The probability that juvenile mosquitoes emerged into adults was significantly affected by the amount of food availability and by the interaction between food availability and parasite infection in the current condition (Table 5.1, Fig. 5.3a-c). It was generally lower for parasitized mosquitoes and under food restriction. Parasites and food during evolution had no significant effects for the probability of emergence but the interaction between them was close to statistically significant. Additionally the three-way inter-

action between parasite infection, food and food during evolution was marginally not significant. Tukey's HSD *post hoc* between coevolution and constant parasite treatments showed no significant differences (analysis not shown).

The adult longevity was significantly lower for infected individuals and with low food availability (Fig. 5.3d-f). Parasite infection, food availability and its interaction had highly significant effects in determining longevity. Parasites during evolution had a close to significant effect in determining longevity and a significant effect in affecting longevity in combination with parasite infection and also with food availability. The food availability during evolution had no significant effects for the adult longevity, but it influenced the longevity in combination with parasites during evolution. The coevolved lines generally lived longer compared to lines kept on constant parasites (Fig. 5.3f). Tukey's HSD *post hoc* tests between coevolution and constant parasite treatments revealed significant differences in adult longevity ($z = 2.64$, $p = 0.022$).

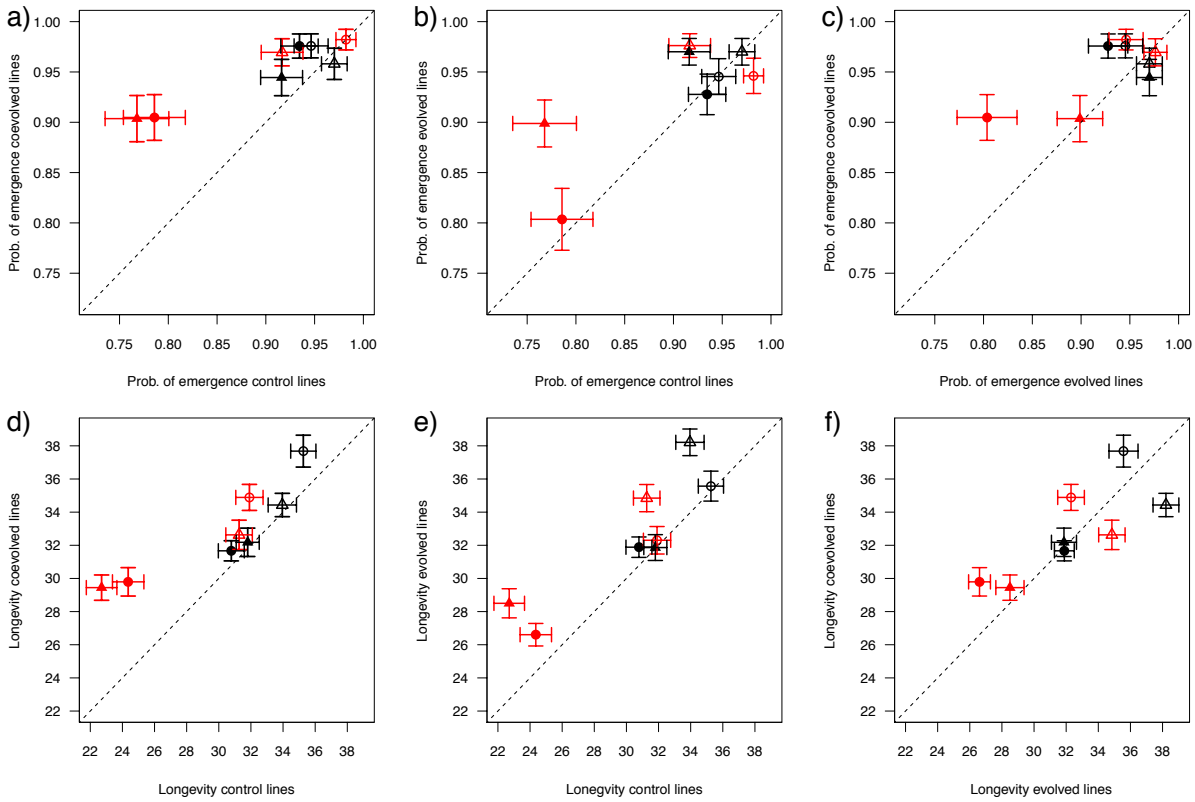

Figure 5:3 Probability of emergence and spore load

Mean probability of emergence of (a) coevolved versus control lines (no parasite), (b) constant parasite versus control lines and (c) coevolved versus constant parasite lines. Mean longevity of (d) coevolved versus control lines, (e) constant parasite versus control lines and (f) coevolved versus constant parasite lines. Every symbol indicates a pairwise comparison of a single treatment between lines from different origins. Triangles represent lines that had low food availability during evolution; squares represent treatments with high food availability during evolution. Red symbols represent treatments that were infected with V.culicis, black symbols represents treatments that were not infected. Open symbols represent treatments with high food availability as larvae; filled symbols represent treatments with low food availability. The dashed line indicates the expectation if mortality, respectively longevity would be equal for control, coevolved and constant parasite lines. Individuals from different replicates are pooled.

Table 5:1 Statistical summary life history traits

Statistical summary for the hosts' life history traits. Generalized linear mixed effect model (binomial distribution) for differences in probability of emergence, mixed effect survival analysis (cox proportional hazard) for age at pupation, linear mixed effect model for wing length and mixed effect survival analysis (cox proportional hazard) for differences in longevity. Statistically significant values are given in bold.

		Probability of emergence		Age at pupation		Wing length		Longevity	
Factor	df	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Parasite	1	0.38	0.538	133.67	**< 0.001**	0.02	0.896	104.49	**< 0.001**
Food	1	9.95	**0.002**	1446.75	**< 0.001**	241.62	**< 0.001**	244.14	**< 0.001**
Evolution parasite	2	2.91	0.233	7.94	**0.019**	1.46	0.483	4.88	0.087
Evolution food	1	1.00	0.317	2.38	0.123	0.01	0.919	0.00	0.968
Parasite x food	2	7.34	**0.007**	40.09	**< 0.001**	5.60	0.018	10.44	**0.001**
Parasite x evolution parasite	2	0.12	0.943	18.52	**< 0.001**	3.11	0.211	10.69	**0.005**
Food x evolution parasite	2	1.02	0.601	6.94	0.031	3.40	0.183	6.16	**0.046**
Parasite x evolution food	1	0.03	0.862	6.37	0.012	1.12	0.290	0.35	0.557
Food x evolution food	1	0.91	0.339	8.60	0.003	0.01	0.917	2.20	0.138
Evolution parasite x evolution food	2	5.14	0.076	3.84	0.146	3.86	0.145	9.57	**0.008**
Parasite x food x evolution parasite	2	0.11	0.949	0.26	0.878	2.36	0.308	7.73	**0.021**
Parasite x food x evolution food	1	3.04	0.081	4.01	0.045	0.48	0.489	2.16	0.141
Parasite x evolution parasite x evolution food	2	2.52	0.283	5.76	0.056	6.35	0.042	1.29	0.526
Food x evolution parasite x evolution food	2	0.28	0.871	4.06	0.131	7.21	0.027	3.40	0.183
Wing length	1	-	-	-	-	-	-	1.60	0.206

5.4 Discussion

Parasitism and the food availability during evolution altered the host's life history in many ways. Mosquitoes originating from lines that were exposed to parasitism during evolution pupated earlier compared to control lines. These results are in accordance with the prediction that earlier reproducing hosts will evolve to reduce the impact of parasitism (Hochberg et al. 1992; Forbes 1993; Perrin and Christe 1996). This was particularly the case when mosquitoes were exposed to infection and under food restriction. In other words, the parasite-induced selection over 10 generations shortened the mosquitos genetically underpinned development time. To our knowledge, this is the first experimental evidence showing parasite infection leads to early reproducing hosts. The shift towards earlier maturation we observed here, might indeed reduce the costs of infection as with ongoing time *V.culicis* proliferates and produces damaging spores (reduced reproductive success and reduced longevity of female mosquitoes (Reynolds 1970, Zeller & Koella, *in prep*)).

The shift had no negative consequences for the adult body size. Accordingly, mosquitoes that were faced to parasitism during evolution are able to grow faster, when parasitized. As the level of parasites virulence is at least partly determined by the genetic basis of mosquitos age at pupation (Koella and Agnew 1999), early emerging individuals were under positive selection when exposed to *V.culicis.* Similarly, the probability that mosquitoes emerged into adults was higher for mosquitoes originating from lines that were faced to parasitism during evolution (when parasitized and under food restriction (Figure 5.3a&b)). In addition to that mosquitoes originating from lines that were faced to parasites during evolution generally lived longer, again especially under parasite exposure and food restriction (Figure 5.3d and 5.3e). These results indicate, that the evolved early maturation seems not to be traded-off with the mosquito's body size or the adult longevity. Mosquitoes that were exposed to parasites during evolution generally showed a shorter development time, an equal body size and a longer survival compared to mosquitoes originating from control lines. It therefore seems that *V.culicis* exerts a general and directional selection pressure on life histories of *Aedes aegypti*. However, we do not know whether *V.culicis* directly exerts selection on the mosquitos age at maturity, longevity or reproductive success, or whether this is the result of selection on a correlated trait.

We found that coevolving parasites did not influence the host's development time differently than constant parasites. However, the duration of the experiment might have been too short to find such differences. A striking result was, that individuals originating from most of the coevolved lines lived longer compared to mosquitoes from lines with constant parasites. One explanation could be, that the evolutionary rate of change in the host's longevity was accelerated by co-evolving parasites (evolutionary arms race). However, when food availability during evolution was low and when tested at high food

levels, the mosquitoes from lines with constant parasites lived longer. This pattern was consistent with and without parasites. Restricted resources therefore seem to impede the host's ability to adapt against co-evolving parasites. Accordingly, co-evolution against *V.culicis* parasites might be costly.

In addition to the evolved adaptations in life histories discussed above, the ecological setting during evolution also affected the mosquito's phenotypic plasticity. The environment in that they were tested explained the biggest part of the phenotypic plasticity (development time and wing length). Still we found enough genetic variability in phenotypic plasticity to evolve under different ecological settings. For example, coevolved mosquitoes that had ample resources during evolution show the smallest plastic response in wing length when faced with parasitism. Accordingly co-evolution might have increased the host's adaptive ability to deal with parasites. However, the trends we describe here are very difficult to interpret and might be influenced by epistasis and pleiotropy (Lynch and Walsh 1998). Nevertheless they illustrate the complexity and dependence from environmental condition of the hosts' life history evolution.

Chapter 6 Synthesis and future research

6.1 Summary of results

This thesis shows that environmental variability can influence many aspects of host-parasite interactions. In the second chapter I describe the effect of variable food availability during the development of the mosquito *Aedes aegypti* on its life-history. One of our questions is whether 'compensatory growth' after a period of undernourishment, which generally appears to be thought of as an adaptive response (Dmitriew 2011), increases reproductive success. We show, however, that compensatory growth did not increase reproductive success. Moving from high to low food availability also had unexpected consequences, leading to lower reproductive success than consistently badly nourished individuals. Varying nutrition is thus clearly important to understand population ecology and life-history evolution. I think that life-history theory should be extended to include these long-term effects of early nutrition. These results are also important because such effects of early nutrition, that alter adult traits, can influence the mosquito's capacity to transmit vector born diseases (Sumanochitrapon et al. 1998; Alto et al. 2008). Accordingly, our data may be useful for predicting disease transmission and developing strategies for mosquito population control.

In the third chapter we study the effect of the environment on the relationship between the growth of the microsporidian parasite *Vavraia culicis* and the longevity of its hosts, the mosquito *Aedes aegypti*. Our data suggest that, in most environments we study, there is a negative relationship between parasite development and host health. However we show that food availability and age at infection can change the effect of parasite growth on host longevity. Such context-dependent relationship between parasite development and virulence can have a considerable impact on the evolutionary outcome of infectious disease. Accordingly, the ecological conditions will change the relative costs and benefits of parasite replication and are likely to determine adaptive levels of virulence. Therefore, theoretical stud-

ies that predict the evolution of virulence should consider, in addition to how the environment affects epidemiologically relevant parameters, also how it affects the relationships between them.

In a further step, we test how the ability of *Ae. aegypti* mosquitoes to resist and tolerate the *V. culicis* parasite evolves in different environments. Unsurprisingly, tolerance and resistance to disease both increased if mosquitoes were exposed to parasites. However, in different evolutionary scenarios, different combinations of these two defense strategies evolved, and in different ecological settings their expression varied. For example, we found that lines that had evolved with low food had higher resistance than those evolved with high food, but there was no difference in tolerance. When we tested the evolved mosquitoes, those that were given restricted food had lower tolerance than those given normal food, but there was no difference in resistance. Such findings illustrate the importance of incorporating environmental heterogeneity, condition dependent evolutionary costs and non-independence between both traits when predicting the evolution of tolerance and resistance (similarly to (Carval and Ferriere 2010)). Our study also provides an example of how resources and the ability to tolerate parasites might interact to determine the evolution of resistance. Such findings are also clinically relevant and might help to elucidate the evolutionary implications of tolerance and resistance based therapies. More broadly this chapter should help to increase our knowledge of the environmental and genetic sources of variation in tolerance and resitance, and how these variations affect fitness. Considering variable ecological conditions is thus clearly important to undersand host-parasite co-evolution.

Food availability and parasite infection also influence the evolution of the hosts' life history. We show that parasite-induced selection over 10 generations can shorten the hosts genetically determined development time. This is in accordance with life-history theory, which predicts that early maturing hosts have fitness benefits when exposed to parasitism (Hochberg et al. 1992). To our knowledge this is the first experimental evidence showing parasite infection leads to the host reproducing earlier than when there is no infection. Mosquitoes that were exposed to parasites during evolution had shorter development times, an equal body size and even a longer longevity compared to mosquitoes originating from control lines. This suggests no evident trade-offs between the traits we measured. The microsporidian parasite therefore seems to exert a general and directional selection pressure on the host's life histories. However, the fact that environmental conditions during evolution and co-evolution had many effects in the expression of the host's life-history traits illustrates the complexity of host-parasite co-evolution. Our results further suggest, that variable environments may influence the long-term host evolution.

6.2 Future directions

In chapter two we describe the reproductive burden associated with compensatory growth. A next step could be to quantify costs of fast growth by comparing oxidative stress and physiological parameters (carbohydrates, lipids, proteins) between fast growing and normally growing individuals (triggered by food availability or temperature). Similar tests could be performed to explore whether parts of the parasites virulence are caused by a parasite-induced increase in the total metabolic rate, which increases the production of free radicals, cellular damage and accelerates ageing processes (van Leeuwen et al. 2010).

Another open question concerns how environmental heterogeneity influences host-parasite dynamics. Environmental conditions can vary strongly across the host's habitat, and hosts within a population are unlikely to all experience the same conditions. It has therefore been argued that environmental heterogeneity may affect the genetic diversity of hosts and parasites (Wolinska and King 2009), the severity of disease outbreaks and virulence (Duffy et al. 2012). Such expectations could be tested with experimental evolution. Disease characteristics could be compared between parasites originating from either evolutionary lines, where the co-evolved hosts received a standard amount of food, or from lines where the co-evolved hosts received variable amounts of food.

In experimental studies of tolerance, the most commonly used measurement of parasite burden is the peak parasite density. This is done because parasite load increases with time after infection and is therefore auto-correlated with longevity and intrinsic mortality rate. However, such measurements miss the early phase of infection. A further step could be to incorporate the growth of the parasite into measurement of tolerance. One possible approach could be to estimate how the probability of dying (at different time points after infection) is influenced by the difference in parasite load between naturally dying and living hosts. Such measurements are further relevant as resistance and tolerance are expected to change throughout an individual's life (Lough et al. 2015).

As described in chapter four, tolerance and resistance can be correlated and increase as an evolutionary response to parasite infection. Studying, whether these trait covariances are the result from adaptive responses to physiological, environmental, or epidemiological factors or whether they result from genetic linkage (pleiotropy, linkage disequilibrium or epistasis) could be a next step. Quantitative analysis of such genetic covariance is, as far as I know, still lacking and could be investigated by ecological

genomics. Theoretical models could be applied to identify situations in which genetic covariation should have strong co-evolutionary consequences.

Additionally, regions in the genome with ecologically relevant functions, that play an important role in the evolution of tolerance, could be tracked. This could illuminate the genetic architecture of evolutionary transitions between antagonism, commensalism and even mutualism.

6.3 Conclusion

Overall this thesis has shown that the environment can influence many aspects of host-parasite interactions, ones that play important roles in shaping evolutionary dynamics. The topics that have been covered are of relevance for several main areas in evolutionary ecology, including life history evolution, epidemiology and resource ecology, and have implications for future research in these fields. By considering that environmental conditions can vary drastically across the host's habitat, and because "the only thing that is constant is change" (Heraclitus, ~ 500 BC), the knowledge presented in this thesis must be considered to fully understand host-parasite interactions and their co-evolution. It also must be incorporated when predicting parasite evolution and especially when managing parasites. The results presented here contribute towards a better understanding of host-parasites interactions, but many questions remain.

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