From gametes to species:
Genetic and non-genetic effects in parasite-mediated selection

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Christian-Albrecht-Universität zu Kiel

vorgelegt von

Joshka Kaufmann

Kiel, 2015

Erster Gutacher: Prof. Dr. Manfred Milinski

Zweiter Gutacher: Prof. Dr. Thorsten Reusch

Tag der mündlichen Prüfung: 07/04/2015

Zum Druck genehmigt: 07/04/2015

Kiel,

Gez. Prof. Dr. Wolfgang Duschl, Dekan

Natural selection is a mechanism for generating an exceedingly high degree of improbability.

~ Ronald A. Fisher,

Reported by Julian S. Huxley in Evolution in Action, 1953.

Table of content

Summary

Parasites represent one of the most notable ecological forces acting in both natural and sexual selection. Their detrimental effects on host condition and reproductive success can have strong consequences on ecological and evolutionary dynamics. Particularly, host-parasite coevolution can lead to local adaptation which in turn can drive the evolution of reproductive barriers between populations and fuel speciation. As hosts are engaged in an arms race against ever-changing parasites, they rely on effective and consistent ways to transmit defenses to their progeny. The work of my thesis examines these two aspects from the host perspective.

In my first two chapters I focused on the evolution of reproductive barriers in incipient ecological speciation in the three-spined stickleback (*Gasterosteus aculeatus*). I particularly examined the role of ecology and parasites in limiting gene flow and maintaining differentiation between lake and river stickleback populations. Following previous work in this system showing local adaptation and assortative mating preferences based on differences at the Major Histocompatibility Complex (MHC), I investigated further mechanisms of reproductive isolation:

First, I tested if post-copulatory pre-zygotic reproductive barriers (e.g. gametic isolation) occurred during incipient ecological speciation between lakes and rivers. Using replicated populations and *in vitro* fertilization assays in a full-factorial design, I could demonstrate ecotype-specific differences in sperm concentration and velocity. Even though these differences did not translate into ecotypic gamete preference, the results suggest an increased developmental failure of the eggs fertilized by heterospecific males. This implies that genetic incompatibilities may arise early on during ecological speciation.

In my second chapter I examined the role of immune local adaptation to parasites in selection against maladapted migrants. Using a field transplant experiment with wild-caught juvenile sticklebacks, I found strong costs of migration between habitats in terms of survival and condition. I could also show that differences in parasites between lake and river were linked to maladapted responses on the innate and the adaptive immune. Furthermore, evidence for habitat-specific associations between local parasite species and locally selected MHC alleles provided insight into the maintenance of MHC diversity at the metapopulation level.

In my final chapter I examined another aspect of the role of parasites, namely the role of paternal non-genetic effects of infection on life-history traits, resistance and tolerance. Until recently, non-genetic transgenerational effects mostly focused on mothers, whereas this experiment demonstrated that infected fathers could also contribute to variation in offspring phenotype. I showed paternal effects of experimental parasite infection mediated through sperm deficiency. By testing and revealing costs and benefits associated with paternal infection and controlling for genetic factors, I provided evidence for the adaptive value of non-genetic transgenerational effects of infection. Furthermore, I showed that resistance and tolerance to parasites can be shaped by both genetic and non-genetic effects. I discuss that the existence of non-genetic effects increasing offspring tolerance but not resistance can have strong consequences on host-parasite dynamics at ecological and evolutionary scales.

This thesis reveals the role of parasites in promoting and maintaining diversity in their hosts, from the level of the sperm phenotype to the species level. While gametic isolation plays a minor role in ongoing ecological speciation, local adaptation to different parasite communities leads to high costs of migration. My work additionally reveals that parasites can affect phenotypic variation not only within but also across generations.

Zusammenfassung

Parasiten sind eine der wichtigsten ökologischen Faktoren, die sowohl in die natürliche als auch sexuelle Selektion eingreifen. Ihre schädlichen Wirkung auf den Gesundheitszustand und den Fortpflanzungserfolg ihres Wirts können sich auf ökologische und evolutionäre Dynamiken auswirken. Vor allem die Coevolution von Wirten und Parasiten kann zur lokalen Adaptation führen, die wiederum zur Ausbildung von Fortpflanzungsbarrieren zwischen Populationen führt und Artspaltung antreibt. Während Wirte sich in einem Wettrüsten mit ständig verändernden Parasiten befinden, brauchen sie auch effektive und zuverlässige Methoden zur Weitergabe von Abwehrmechanismen an ihre Nachkommen. In meiner Doktorarbeit habe ich diese beiden Aspekte näher untersucht.

In den ersten beiden Kapiteln habe ich mich auf die Evolution der Fortpflanzungsbarrieren in den frühen Stadien ökologischer Artbildung in Dreistachligen Stichlingen (*Gasterosteus aculeatus*) konzentriert. Ich habe ins Besondere die Rolle ökologischer Faktoren und von Parasiten auf die Einschränkung von Genfluss zwischen See- und Fluss-Populationen und deren Differenzierung untersucht. Frühere Arbeiten haben lokale Anpassungen und assortative Paarung nachgewiesen, die durch Unterschiede im Haupthistokompatibilitätskomplex (Major Histocompatibility Complex, MHC) in Stichlingen hervorgerufen wurden, woraufhin ich weitere Mechanismen der reproduktiven Isolation in diesem System untersucht habe:

Als erstes habe ich überprüft, ob postkopulative, präzygote Fortpflanzungsbarrieren (z.B. gametische Isolation) zwischen See- und Fluss-Populationen bei einsetzender ökologische Artspaltung existieren. Mit replizierten Populationen und *in vitro*-Tests konnte ich Ökotyp-spezifische Unterschiede in Spermienkonzentration und -geschwindigkeit nachweisen. Diese Unterschiede haben sich nicht in der Gameten-Präferenz innerhalb Ökotypen widergespiegelt aber dafür deuten die Ergebnisse dieses vollfaktoriellen Experiments auf einen erhöhten Ausfall von Eiern während der Entwicklung hin, die durch heterospezifische Männchen befruchtet wurden. Dies bedeutet, dass genetische Unverträglichkeiten schon früh in der ökologischen Artbildung auftreten können.

In meinem zweiten Kapitel habe ich die Rolle von lokaler Immunoanpassung an Parasiten während der Selektion gegen schlecht angepassten Migranten untersucht. Mit einem

Feldversuch mit wild gefangenen, juvenilen Stichlingen fand ich große Fitnessnachteile vonMigration zwischen Lebensräumen in Bezug auf Überleben und Gesundheitszustand. Ich konnte Unterschiede zwischen Parasiten in See und Fluss mit schlecht angepassten Antworten des angeborenen und adaptiven Immunesystems in Verbindung bringen. Darüber hinaus gaben die Ergebnis zur Habitat-spezifischen Zuordnungen von lokalen Parasitenarten zu lokal selektierten MHC-Allelen einen Einblick in die Aufrechterhaltung von MHC-Diversität auf der Ebene von Metapopulationen.

In meinem letzten Kapitel habe ich einen weiteren Aspekt der durch Parasiten vermittelten Selektion untersucht: die Rolle von paternalen, nicht-genetischen Effekten von Infektionen auf Life-History-Merkmale, Resistenz und Toleranz. Bisher wurden hauptsächlich nicht-genetische, generationsübergreifend Effekte von Müttern untersucht. Das hier beschriebene Experiment hat jedoch gezeigt, dass infizierten Väter auch zur Variation des Phänotyps der Nachkommen beitragen können. Ich konnte zeigen, dass diese paternalen Effekte bei experimenteller parasitärer Infektion durch Spermiendefizite vermittelt wurden. Durch Untersuchung und Aufdeckung der Kosten und Nutzen paternaler Infektion, unter Berücksichtigung genetischer Faktoren, konnte ich Beweise liefern für den adaptiven Nutzen von nicht-genetischen, generationsübergreifenden Effekten parasitärer Infektionen. Darüber hinaus habe ich gezeigt, dass Resistenz und Toleranz gegen Parasiten sowohl durch genetische als auch nicht-genetische Effekte geformt werden können. Ich habe erörtert wie nicht-genetische Effekte, die die Toleranz aber nicht die Resistenz der Nachkommen erhöht, starke Auswirkungen auf die Wirt-Parasit-Dynamik im ökologischen und evolutionären Ausmaß haben können.

Diese Doktorarbeit offenbart die Rolle von Parasiten bei der Förderung und Aufrechterhaltung der Diversität in ihren Wirten, von der Ebene Spermien-Phänotyps bis zur Artenebene. Während gametische Isolation nur eine untergeordnete Rolle bei der andauernden ökologischen Artbildung spielt, führt die lokale Adaptation an verschiedene Parasitengemeinschaften zu hohen Kosten bei Migration. Meine Arbeit zeigt darüber hinaus, dass Parasiten phänotypische Variation nicht nur innerhalb einer Generation sondern auch generationsübergreifend beieinflussen können.

Introduction

Understanding the origin and the maintenance of biodiversity lies at the root of all questions in evolutionary biology. Evolutionary biologists have strived to understand the proximal and ultimate causes for variation within and between species. Particularly, among the still unanswered questions lies the role of ecological forces on the evolution of adaptive traits and on the formation of species. Parasites could be a key to solve these questions as they represent a strong selective force, ultimately driving the evolution of host traits and maintaining polymorphism through reciprocal adaptation.

In the general introduction of my Ph.D. thesis, I will first explain how parasites represent one of the most notable ecological and evolutionary forces in both natural and sexual selection. By describing the state-of-the-art of research on parasite-mediated selection, I will provide the motivations underlying my studies. I will then focus on the role of parasites in the evolution of reproductive barriers leading to ecological speciation.

1.1. Parasite-mediated selection

A parasite is defined as an organism living in or on another living organism, obtaining resources from its host (e.g. nutrients) and causing various degrees of damage (Poulin 2006). Parasites are remarkable agents of selection as they increase their own fitness at the expense of the host's resources and reproductive success (Maynard Smith, 1978). Parasitism is a very successful strategy, as it has been suggested that parasites greatly outnumber free living organisms (Windsor 1998; Kuris *et al.* 2008) and that there are probably no parasite-free organism (Poulin 1996).

The constant coevolutionary struggle between hosts and parasites can help explain a great mystery: the maintenance of sexual reproduction (Hamilton 1980; Hamilton *et al.* 1990). Although asexuals have a two-fold reproductive advantage - they reproduce at twice the rate of sexuals - sex is widespread throughout plant and animal kingdoms (Maynard Smith 1978). The maintenance of sexual reproduction can be explained by an advantage linked to recombination during the dynamic race between hosts and parasites. The Red Queen hypothesis suggests that "for an evolutionary system, continuing development is needed just

in order to maintain its fitness relative to the systems it is coevolving with" (Van Valen 1973). Sex can thus provide a benefit in a changing environment (Jaenike 1978; Moritz 1991; Becks & Agrawal 2012).

1.1.1. Host-parasite dynamics

Hosts and parasites are engaged in an arms race for survival. The selection that each antagonistic actor imposes on the other thus causes strong reciprocal adaptation. This is illustrated by complex dynamics of allele frequency changes, for alleles coding for host resistance and parasite infectivity (Ebert & Hamilton 1996; Little 2002; Jokela *et al.* 2009). These dynamics can take different forms: (i) recurrent adaptation, where advantageous alleles sweep through host and parasite populations, leading to the repeated fixation of virulence and defense traits (e.g. Poullain *et al.* 2008); (ii) Red Queen dynamics *sensu stricto*, characterized by negative-frequency-dependent selection (e.g. Koskella & Lively 2009). In the latter, host and parasites adapt to common antagonistic genotypes, leading to out-of-phase fluctuations in allele frequencies. These adaptive changes rely on standing genetic variation and the recycling of alleles. Notably, allele frequencies can increase from both rare or frequent advantageous alleles (Lively & Dybdahl 2000; Bernatchez & Landry 2003; Jokela *et al.* 2009; Eizaguirre *et al.* 2012a).

Although demonstrating such patterns of ongoing selection is not straightforward, the recent use of experimental (co)evolution experiments has permitted to empirically test the Red Queen hypothesis. In addition to the seminal work of Curtis Lively (1987) that showed correlation between rates of sexual reproduction and parasite resistance in *Potamopyrgus* snails (Lively 1987), recent experimental work has confirmed that rapid antagonistic coevolution leads to both an advantage of sex and an increase in genetic diversity: Using the host nematode *Caenorhabditis elegans* and its parasite the bacteria *Bacillus thuringiensis*, Schulte and colleagues (2010) experimentally showed that antagonistic coevolution followed rapid temporal genetic fluctuation. There, Red Queen dynamics increased host resistance and parasite virulence and ultimately led to high genetic diversity. In an experiment "resurrecting" dormant stages of *Daphnia* and their bacterial endoparasites, Decaestecker and colleagues (2007) could reproduce the patterns of evolution and reciprocal adaptation across time, see also (Gandon *et al.* 2008). Lastly, a recent selection experiment has shown that one generation

is sufficient to select for increased parasite species-specific resistance via adaptive change in immunogenetic allele frequency (Eizaguirre *et al.* 2012a). Those fascinating examples represent only a fraction of the efforts to reveal the role of parasites in the maintenance of sex (Morran *et al.* 2011), of genetic diversity (Koskella & Lively 2009; Paterson *et al.* 2010; Schulte *et al.* 2013) and in coevolutionary dynamics of both host and parasite traits (Dybdahl & Lively 1998; Jokela *et al.* 2009; King *et al.* 2011). As the Red Queen hypothesis relies on variation in host defenses, I will present the diversity of adaptive defenses against parasites and their mode of inheritance in the next two sections.

1.1.2. Defenses against parasites

The diversity of parasites that hosts can encounter has led to the evolution of a diverse range of defenses. These include behavioral avoidance as well as physical, cellular or molecular barriers (Janeway 2001; Moore 2002; Schulenburg *et al.* 2009). Particularly, complex immune systems have evolved to limit both (i) levels of infection via resistance and (ii) associated damages via tolerance.

Resistance and tolerance are two alternative but complementary strategies. Until recently, the subtle semantic distinction has led to misunderstandings in parasitology and evolutionary ecology (Medzhitov *et al.* 2012). Resistance is defined as the protection of the host to avoid reaching high levels of parasite infection, by limiting parasite contact, entry and establishment in or on the host. Tolerance reduces the deleterious consequences of parasite infection - the host withstands infection but suffers limited fitness consequences (Svensson & Råberg 2010). Resistance and tolerance can be disentangled by looking at (i) differences in infection rates (i.e. standardized parasite burden) and (ii) differences in fitness with similar parasite burden respectively (Fig. 1). Thus, increased Darwinian fitness can theoretically be achieved by higher resistance associated with lower parasite burden and/or by higher tolerance associated with higher costs' mitigations. Recently, Råberg and colleagues (2007)established a statistical framework, derived from plant-pathogen research, to identify differences in resistance and tolerance between groups (e.g. strains, experimental treatments, populations or species) (Råberg *et al.* 2007). There, parasite resistance is estimated as the inverse of parasite burden and tolerance as the rate of change in fitness as parasite burden increases (see Figure 1) (Ayres & Schneider 2012; Vale *et al.* 2014).

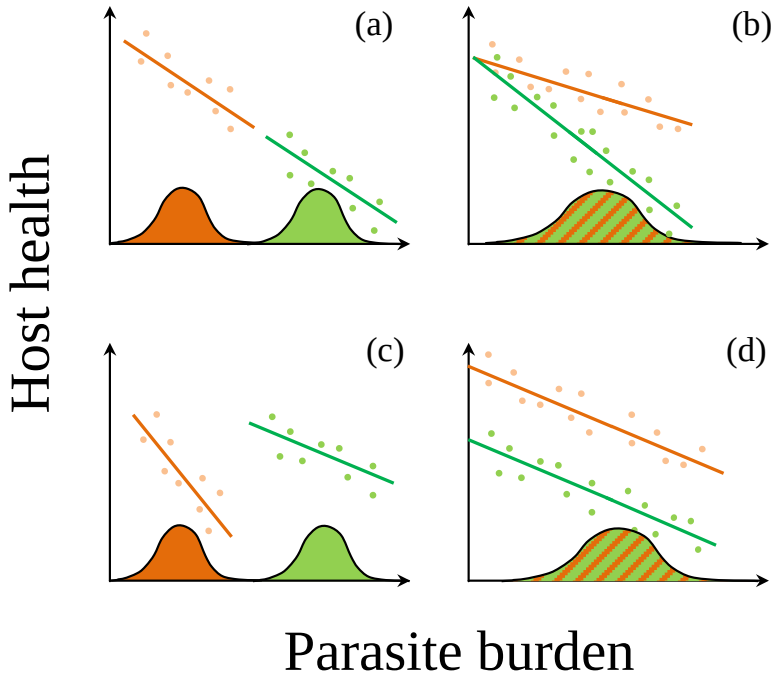


Figure 1: Disentangling parasite tolerance and parasite resistance: Tolerance is defined as the reaction norm of health over the level of infection (i.e. slopes), whereas resistance is defined as the average parasite burden. The green and orange colors represent two different groups (e.g. genotypes, populations, species). Thus, differences in slopes between groups indicate differences in tolerance whereas differences in mean parasite burdens indicate differences in resistance. (a): same tolerance, difference in resistance ($R_{orange}>R_{green}$); (b): difference in tolerance ($T_{orange}>T_{green}$), same resistance ; (c) differences in both tolerance and resistance (higher tolerance for green, higher resistance for orange); (d) no differences in either tolerance or resistance but a discrepancy in vigor (higher mean health for orange). Figure adapted from Råberg 2007.

On one hand, resistance involves surface, cellular and extracellular barriers. On the other hand, tolerance involves tissue repair (Playfair *et al.* 1990), the limitation or control of "overly exuberant immune responses" or immunopathology (i.e. pathology associated with immune responses against the pathogen) (Råberg *et al.* 2009; Sorci 2013). For example, high levels of tolerance can be achieved by the production of anti-inflammatory cytokines by regulatory lymphocytes, leading to the resolution of a costly inflammatory response and a return to a healthy homeostatic state (Long *et al.* 2008a; b; Belloni *et al.* 2010).

When studying evolution of host defenses, it is important to distinguish between these two processes as they can have different consequences on pathogen and host phenotypes as well as ecological and evolutionary dynamics (Roy & Kirchner 2000). Host-parasite interactions

involving resistance lead to antagonistic coevolution, as an increase in resistance, by definition, reduces the reproductive success of non-adapted parasites and thus parasite population size (Svensson & Råberg 2010). However, increase in tolerance does not limit the establishment of parasites but mainly reduces their impact on the host fitness and thus, theoretically, has no negative effect on parasite population size and reproductive success (Miller *et al.* 2005). Models even showed that, by imposing mild selective pressure on pathogens, tolerant hosts can potentially act as pathogen reservoirs, increasing the overall transmission and ultimately the fitness of the pathogen (Roy & Kirchner 2000; Miller *et al.* 2005; Best *et al.* 2014).

Immune systems

Immune defenses play a central role against parasite infection and consequent pathologies. The immune system is commonly defined as the biological processes recognizing non-self from self, leading to the protection against diseases. The vertebrate immune system can be seen as two independent albeit intertwined parts: the innate and the adaptive immune system, based on both humoral and cell-mediated immunity.

The innate immune system incorporates rapid but rather unspecific responses against all pathogens. These first barriers to infection include surface and cellular barriers, inflammatory responses characterized by the recruitment of immune cells and biochemical cascades such as the activation of the complement system (Janeway 2001). The immune cells involved in this immediate response to infections include granulocytes (basophils, eosinophils and neutrophils), macrophages, dendritic cells and natural killer cells. The innate immune system reacts in an antigen-independent manner, meaning that responses are thought to be rather unspecific and can induce immunopathological costs (e.g. inflammation) at each infection. Immunopathology represents host damage following immune activation. Noteworthy, recent studies show a certain level of pathogen-specificity of the innate immune system relying for example on germline-encoded pattern-recognition receptors (Kurtz & Franz 2003; Schmid-Hempel & Ebert 2003).

The adaptive immune system, found only in jawed vertebrates, is characterized by a delayed response to infection and a high specificity due to being antigen-dependent. It is based on recognition of antigens (*anti*body *gen*erators) by immune cells and immunological memory after exposure to pathogens. Non-self antigens can be foreign molecules that stem from foreign bodies (e.g. viruses, bacteria, and macroparasites). After binding to immune receptors

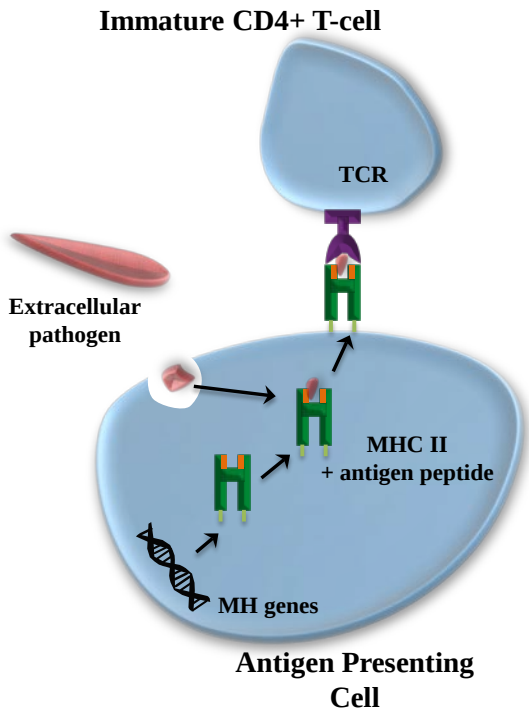
which are present on most cells, antigens are presented to T-lymphocytes (Box 1). Thereafter, a variety of responses are initiated to eliminate the identified pathogens or pathogen-infected cells: proliferation of T-lymphocytes, recruitment of granulocytes, cytotoxicity by $CD8^+$ T lymphocytes, production of memory B cells and antibodies. These responses provide an extremely efficient way to fight specific pathogens. Amongst others, Major Histocompatibility Complex (MHC) molecules play a central role in specific immunological responses (Janeway *et al.* 2001).

The proximate and ultimate roles of MHC

MHC surface molecules present peptide antigens to T-lymphocytes, inducing immunological recognition between self and non-self (see Box 1). MHC Class I molecules bind specifically intracellular pathogens (e.g. viruses) and MHC Class II bind extracellular pathogens (e.g. macroparasites). Responses of the adaptive immune system (cell death, macrophage activation, B cell activation) are thus highly dependent on the variability of MHC genes coding for these molecules. It is not surprising that MHC genes are amongst the most diverse genes in the genome in terms of copy number variation (e.g. Chain *et al.* 2014), allelic diversity (Tennessen *et al.* 2012) and sequence divergence (Kelley *et al.* 2005). In humans for example, over 9200 alleles were found at MHC class I loci and 3000 at MHC class II (Robinson *et al.* 2013). This rare level of polymorphism is thought to be maintained by balancing selection on MHC genes as a result of parasite-mediated selection.

Several hypotheses have been proposed to explain the maintenance of such polymorphism, such as heterozygote advantage (Doherty & Zinkernagel 1975), negative-frequency dependent selection (Clarke & Kirby 1966; Borghans *et al.* 2004; Eizaguirre *et al.* 2012a) and fluctuating selection (Hill 1991). Although these hypotheses are not mutually exclusive (Apanius *et al.* 1997; Milinski 2006; Eizaguirre *et al.* 2009b; Lenz *et al.* 2009b), the relative contribution of each in the maintenance of MHC polymorphism is still under debate (Slade 1992; Potts & Slev 1995; Edwards & Hedrick 1998). Specific MHC alleles or an optimal diversity can be (i) naturally selected through a direct selective advantage in resistance to pathogens (Wegner *et al.* 2003a) and (ii) sexually selected by female preference for males carrying specific alleles ("good genes") or an optimal number of alleles (Milinski *et al.* 2005; Eizaguirre *et al.* 2009b; Kamiya *et al.* 2014).

Box 1: The MHC II pathway



Genes of the Major Histocompatibility Complex class II code for surface molecules involved in antigen presentation to immature helper T lymphocytes. MHC class II proteins (green) are expressed on the surface of antigen presenting cells (e.g. macrophages, B cells or immature dendritic cells). Contrary to MHC class I, MHC class II molecules are specialized in the presentation of protein-derived peptides from extracellular pathogens (bacteria, macroparasites; red). After a protein is endocytosed and degraded, derived peptides will be bound by MHC class II molecules and displayed on the cell surface. The antigen-binding sites located in the peptide-binding groove (orange) are responsible for binding the antigenic peptides. MHC class II proteins present this peptide to the T-cell receptor (TCR, purple) of immature $CD4^+$ helper T lymphocytes. This ultimately triggers T-cell proliferation, cytokine secretion, macrophage activation and antibody production, thus regulating and assisting in the active immune response to extracellular pathogens. The MHC genes coding for the peptide binding region exhibit a high degree of polymorphism, increasing the variety of peptides that can be presented to T cells.

1.1.3. The inheritance of defenses against parasites

The theory of host-parasite coevolution is mostly based on host resistance and pathogen virulence (Anderson & May 1982). To be adaptive, these traits need to provide an advantage but must also be variable and heritable. Heredity is defined as the transmission of characteristics from parents to offspring. Since the Modern Synthesis, evolutionary biologists almost unanimously agreed that heredity relies on vertically transmitted genetic material (Fisher, 1930 chapter I; Jablonka & Lamb, 1995). As we have seen, coevolutionary dynamics rely on reciprocal changes in allele frequencies and therefore on both standing genetic variation and strong genetic basis for host defenses (Malo & Skamene 1994; Sorci *et al.* 1997; Hill 1998) Although host-parasite coevolution theory has been mostly established on a genetic basis of inheritance, it has now been acknowledged that non-genetic components can also play a role in short- and long-term effects.

Non-genetic transmission of host defenses

The recent regain of interest for nongenetic transgenerational effects (i.e. epigenetics, maternal effects) provides a perspective for extending and developing theories in evolutionary ecology of host-parasite coevolution and evolutionary biology in general (Wolf *et al.* 1998; Jablonka 2009; Day & Bonduriansky 2011; Bonduriansky 2012) see Box 2). Under this theory, phenotypic variation -i.e. the target of selection- is not only depending on genes or on the environment but also on influences from the parental phenotype and life history that can be transferred across generations.

The effect of parasite infections can cross transgenerational barriers and affect offspring immunity and fitness drastically (Poulin & Thomas 2008). Several mechanisms can shape an individual's resistance or tolerance against pathogens depending on its parents' parasitic experience. Although transgenerational phenotypic plasticity cannot be defined as inheritance *sensu stricto*, it represents an additive component in the variation of offspring phenotype and defenses (Bonduriansky & Day 2009). Prenatal and postnatal maternal effects of parasite infection can take many forms, such as adjustment of parental care (Mousseau & Fox 1998).

Box 2: Inheritance revisited: A pluralistic model of heredity

One of the pillars of evolution by natural selection is that traits conferring a fitness advantage are heritable (Darwin 1859). After the development of the Modern Synthesis of Evolutionary Biology, heritability was mainly characterized by a universal mechanism, the vertical transmission of genetic material. Although some scientists discussed alternative models of heredity, most 20th century evolutionary theories were established on vertical transmission of DNA or "hard" inheritance. Soft inheritance (for instance Lamarckian inheritance) based on the transgenerational transmission of other components than DNA influenced by parental phenotype and/or environment, was not strongly considered at the time. Robust evidence is now accumulating for mechanisms of non-genetic inheritance, such as maternal effects (Mousseau & Fox 1998), epigenetic marks affecting gene expression (Goldberg *et al.* 2007), somatic inheritance (Skinner *et al.* 2012) or social/cultural inheritance (Laland *et al.* 2010). These mechanisms that contribute to phenotypic variation across generations, have the potential to be adaptive (Marshall & Uller 2007; Burgess & Marshall 2014) and could affect evolutionary dynamics (Klironomos *et al.* 2013a). An emerging extended model of inheritance has been developed to recognize both soft and hard inheritance (Danchin & Charmantier 2011; Bonduriansky 2012). This synthetic theory acknowledges that other factors (green arrow; i.e. information, molecules, resources, territories) are transmitted across generations alongside alleles (blue arrow), while appreciating the fact that the latter are essential. This model represents an exciting new development for ideas in evolutionary biology and provides the potential to revisit previously established theories from a more comprehensive perspective.

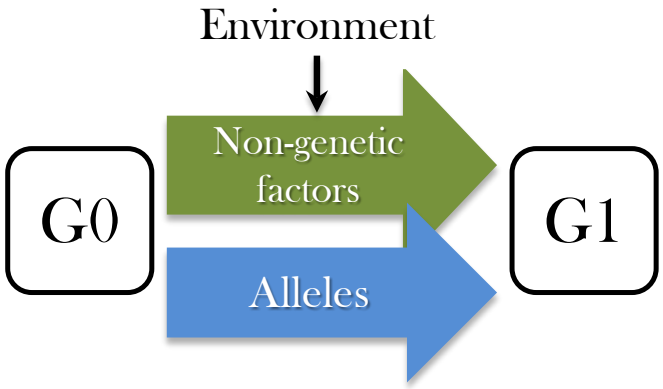


Figure adapted from (Bonduriansky 2012).

When infected or immunologically-challenged, mothers can transfer various molecules through placental, milk, egg yolk or albumen such as nutrients, antibodies (Buechler *et al.* 2002; Hasselquist & Nilsson 2009), hormones (Hayward & Wingfield 2004; Postma *et al.* 2014) or lysozymes (Saino & Dall'Ara 2002).

Transmission of maternal immunity is not a new finding, as it was initially discovered in vertebrates over 120 years ago (Ehrlich 1892). Maternal transfer of antibodies or antibody-like structures is particularly interesting, as it leads to trans-generational immune priming (i.e. trans-generational vaccination effects). This increases offspring defenses during their early life-stages, when the adaptive immune system is naïve and the offspring most vulnerable. Circulating antibodies are transferred as a result of maternal infection or immune activation (Grindstaff *et al.* 2003; Hasselquist & Nilsson 2009). Transgenerational immune priming can increase humoral activity (Lemke *et al.* 2004) and can enhance immune responses from months to years after exposure (Reid *et al.* 2006; Ramos *et al.* 2014).

Research on parental effects focused previously on mothers due to their close physical and physiological link to their offspring. However, the fact that epigenetic marks (e.g. methylation, acetylation patterns) can be inherited allow to go beyond the effect of the sole mother and integrate paternal life-history in shaping offspring phenotype (Morgan *et al.* 1999; Jablonka 2009). Fathers can thus also contribute to variation in offspring immune defenses through paternal effects (Curley *et al.* 2011; Rando 2012). However, discussions on the direct or indirect nature of paternal effect just recently started, making the study of the underlying mechanisms of paternal immune priming a new axis of investigation (Crean & Bonduriansky 2014). The exact nature of the factors involved still represent a mystery, but sperm (i.e. spermatozoa and associated proteins) is an ideal candidate for mediating paternal effects (Crean et al. 2012; Rando 2012, Bromfield et al. 2014). In pipefishes, the evolution of paternal immune priming has been proposed to be associated with the evolution of sex-role reversal (Roth et al. 2012). However, this effect can also occur in "classic" sex-roles species. For example, paternal priming has also been found in the red flour beetle where mechanisms might involve sperm and sperm-associated proteins (Roth et al. 2010; Eggert 2014). These studies however focus on the role of immune activation on transgenerational priming by using heat-shock killed bacteria rather than live pathogens. Consequently, the mechanisms of paternal effects of pathogen infection per se, as well as the costs and consequences of this effect are still largely unknown.

Evolutionary implications of non-genetic transgenerational effects

Transgenerational effects can prepare offspring for a parasite-rich environment and can thus be extremely advantageous when parasite presence is predictable. However, acknowledging such effects as possible adaptive evolutionary responses is quite recent (Marshall & Uller 2007). At this point it is important to clarify the distinction between transgenerational effects that have the potential to be adaptive (i.e. the mechanism of transmitting information or resources) from the information or resources itself. Notably, in many cases, parental infection can induce only costs on offspring fitness and these transgenerational effects do not always represent the product of selection but could only the consequence of physiological or developmental constraints (Gould & Lewontin 1979; Linder & Promislow 2009). Nongenetic transgenerational effects, together with transgenerational changes in allele frequencies (Eizaguirre *et al.* 2012a), can represent a fast response to selection (Marshall 2008). These transgenerational effects can efficiently influence offspring phenotypes in response to prevailing environmental stress. They can thus complement fast changes in allele frequencies, by providing adaptive plasticity to individuals carrying non-advantageous genetic material (Mousseau et al. 2009). Adaptive parental effects are expected to have evolved under predictable and reliable environments across generations so that parents have reliable cues to future conditions (Marshall & Uller 2007; Burgess & Marshall 2014). The strict context-dependence of such effects as well as the high direct costs (due to the presence of the stressor) can explain why not all individuals in a population can express parental effects (Marshall 2008; Uller 2008). Overall, evidence for adaptive parental effects is extremely weak and virtually absent for parasite infections (Uller et al. 2013).

Clearly parasites represent a selective pressure that varies and, within the time frame of successive generations, is to some extent predictable. Indeed, even if not vertically transmitted, parasite infections during the parental generation increase the likelihood that offspring get exposed to the same parasite species and/or genotype. The presence of a parasite in a given generation is thus more likely to predict parasite presence in the next generation than to predict parasite absence in the next generation.

Although non-genetic transgenerational effects are transitory and short-timed, recent models showed that they can ultimately play a significant role in selection and the speed of evolution (Klironomos *et al.* 2013a; McGlothlin & Galloway 2013). Theoretical studies have shown

that transgenerational immune priming, while beneficial in the short term, might increase parasite prevalence in the long term (Mostowy 2012; Tidbury *et al.* 2012). Mostowy and coworkers (2012) suggested theoretically that non-genetic transgenerational effects can completely eliminate coevolutionary oscillations and bring Red Queen dynamics to a stop. Also, by maintaining non-selected alleles in the population through the survival and reproduction of genetically non-resistant individuals, non-genetic transgenerational effects can maintain a high level of immunogenetic diversity. Further theoretical but mostly empirical work is therefore needed to understand the complex interplay between genetic and non-genetic transmission of host defenses and the relative role of non-genetic effects.

1.2. Speciation

1.2.1. On the origin of speciation

Understanding the origin of species is the “mystery of mysteries” of evolutionary biology (Darwin 1859). To understand the conditions and the mechanisms of speciation, we first have to define “species”. The working definition used by most evolutionary biologists is the biological species concept: “groups of interbreeding natural populations that are reproductively isolated from other such groups” (Mayr 1942).

This central focus of early speciation research was allopatric speciation, where physically isolated populations undergo genotypic divergence. Upon secondary contact, individuals from respective populations have become unable to interbreed and thus reproductively isolated (Dobzhansky 1940; Mayr 1963). There are multiple mechanisms of reproductive isolation, including premating isolation, postmating prezygotic barriers (i.e. gametic isolation) and postzygotic barriers (Coyne & Orr. 2004).

Although Mayr recognized the role of ecological divergence in speciation (Mayr 1947), divergence between populations was mostly attributed to neutral processes reducing gene flow (i.e. genetic drift between allopatric populations). Divergence with gene flow- in sympatric and parapatric systems- is what distinguishes recent models from previous models of speciation (Butlin *et al.* 2008; e.g. Niemiller *et al.* 2008). More than 150 years after Darwin, substantial attention is again being paid to the role of natural selection in speciation. Recently, research on ecological speciation has focused on the role of divergent natural selection among different ecological habitats (Schluter & Conte 2009).

1.2.2. Ecological speciation

Ecological speciation occurs when the evolution of reproductive isolation results from divergent selection on ecologically-linked traits in different ecological niches (Schluter 2001; Rundle & Nosil 2005). This leads to the formation of locally adapted and genetically-discrete demes, named ecotypes or ecomorphs, which can be preliminary steps of speciation.

Association between ecological divergence and population differentiation can stem from a linkage of the genes under ecological selection with the ones contributing to reproductive isolation (Hawthorne & Via 2001; Rundle & Nosil 2005; Kronforst *et al.* 2006). Alternatively, this can be achieved by "magic traits" with pleiotropic effects on both natural selection based on ecological contrasts and reproductive compatibility between ecotypes (Eizaguirre *et al.* 2009a; Servedio *et al.* 2011; Servedio & Kopp 2012).

Gene flow between ecotypes continues to decrease as drift and reinforcement contribute to differentiation, potentially resulting in complete reproductive isolation and species formation. Reinforcement is the evolution of additional barriers due to selection against hybridization (Coyne & Orr. 2004). Although much attention has been given to examples where ecological divergence drove sympatric speciation, ecological speciation can occur also in allopatry (fully geographically isolated populations) and parapatry (adjacent populations, with potential gene flow) (Mayr 1947; Wu & Ting 2004). It is interesting to note that finding evidence for ecological speciation in sympatric systems provides support for ecological speciation in any of these contexts. Overall, finding evidence of reproductive barriers in sympatric and parapatric system is particularly interesting, as potential gene flow in these systems is predicted to impede adaptation (Garant *et al.* 2007).

Although many reviews of ecological speciation have been written (Rundle & Nosil 2005; Schluter 2009; Nosil *et al.* 2009; Hendry 2009; Boughman 2013), comprehensive empirical studies are surprisingly rare. Hendry (2009) suggested that studied systems do not always show signatures of ecological speciation because adaptive divergence is not always associated with reproductive barriers. Ecological speciation is a continuum with overlapping following stages of adaptive variation, adaptive divergence and various levels of reproductive isolation (Hendry 2009; Theis *et al.* 2014). Systems with strongest evidence for adaptive radiation based on ecological factors include fishes (Schluter 1996; McKinnon & Rundle 2002; Barluenga *et al.* 2006; Eizaguirre & Lenz 2010), phytophagous insects (e.g. Via *et al.* 2000) and species restricted to islands (e.g. Losos & Ricklefs 2009; Losos & Mahler 2010).

There has been substantial recent progress in understanding the genetics mechanisms underlying ecological speciation. Investigating genome-wide patterns of genetic variation associated with ecological speciation revealed (i) loci under divergent selection showing high levels of differentiation between ecotypes (Hawthorne & Via 2001; Renaut *et al.* 2012; Feulner *et al.* 2015) and (ii) the genetic architecture of the phenotypes involved (Peichel *et al.*

2001; Rogers & Bernatchez 2007; Arnegard *et al.* 2014). Cases of repeated evolution between ecotypes highlight the potential importance of recurrent evolution based on standing genetic variation. For example, Jones and colleagues (2012) show the repeated use of the same loci and chromosomal inversions in differentiation between marine and freshwater stickleback population pairs across the Northern Hemisphere.

Despite recent theoretical and empirical advances on the role of ecology in population differentiation and speciation, we still lack understanding of the mechanisms responsible for the evolution of reproductive isolation (Gavrilets 2003; Butlin et al. 2012). Particularly in cases of divergence with gene flow, research is needed to explain when and how barriers form and how different barriers work in concert. Functional links must be made between genotype, phenotype, fitness and reproductive isolation (Smadja & Butlin 2011; Butlin *et al.* 2012). Although genomic tools might help us understand the underlying genetic basis of ecological speciation, identifying which ecological factors are responsible for adaptive divergence among populations remains of primary importance.

1.2.3. Parasite-mediated speciation

Parasites and pathogens represent ubiquitous ecological pressure strongly acting on host natural and sexual selection. Due to high specificity between host and parasite genotypes, it has been suggested that parasites can play a major role in initiating or reinforcing reproductive barriers in hosts throughout the speciation process (Price *et al.* 1986; Haldane 1992). In the following section, I will explain how host-parasite coevolution can fuel speciation processes.

Parasite-mediated speciation requires (i) differences in parasite communities between habitats and (ii) local adaptation of the host within habitats (Eizaguirre *et al.* 2009a; Eizaguirre & Lenz 2010; Karvonen & Seehausen 2012). Discrepancies in parasites distribution between ecotypes has been reported in a number of taxa (reviewed in Karvonen & Seehausen 2012). These differences can result from various factors, such as abiotic factors or the presence of intermediate hosts (Poulin 2006). The differences in parasite abundance or communities between habitats are likely to generate genetically locally adapted host populations (Eizaguirre & Lenz 2010; Eizaguirre *et al.* 2012b). Notably, although habitat differences in the abundance of one parasite species might cause population differentiation (De Roij *et al.*

2011), the joint effect of multiple parasites is more likely to drive speciation (Eizaguirre *et al.* 2011).

Parasite-mediated natural selection in ecological speciation

Higher parasite loads in migrants and hybrids as a result of local adaptation can impose strong costs on condition and survival, which ultimately reduce fitness of migrants and hybrids (Kaltz & Shykoff 1998; Thompson 2002; Nosil *et al.* 2005). Evidence for parasite-mediated natural selection acting against maladapted hosts in the context of speciation is mostly correlative and largely inconclusive. Some studies have shown higher parasite loads in hybrids, and other lower parasite loads (Sage *et al.* 1986; Fritz *et al.* 1999; MacDougall-Shackleton *et al.* 2002). However, a few transplant experiments between subpopulations from different ecotypes have shown that local adaptation can result in reduced condition of migrants or hybrids (MacColl & Chapman 2010; Eizaguirre *et al.* 2012b; Räsänen & Hendry 2014). These costs can be mediated by divergence in adaptive immune genes and divergence in the expression of innate and adaptive immune responses (Rauch *et al.* 2006; Scharsack *et al.* 2007; Eizaguirre *et al.* 2012b).

Parasite-mediated sexual selection in ecological speciation

Parasite-mediated sexual selection can also play a role in species divergence. Parasites affect sexually selected traits (i.e. ornaments), and females have evolved preference towards resistant males characterized by "flamboyant" ornaments (Darwin 1871; Zahavi 1975; Hamilton & Zuk 1982; Milinski & Bakker 1990). In the context of local adaptation, mate preference for highly resistant and tolerant individuals selects against locally maladapted hosts with higher parasite load and low condition. Parasite-mediated sexual selection can thus directly contribute to the maintenance and acceleration of habitat-specific population differentiation and play a role in reinforcement of local adaptation (Ritchie 2007; Eizaguirre *et al.* 2009a, 2012b; Maan & Seehausen 2011). Correlative studies have shown associations between the level of parasite infection and variation in sexually selected traits (i.e. coloration) within populations (Moller *et al.* 1999; Maan 2006; Maan *et al.* 2008). Causal evidence for the role of parasite-mediated sexual selection in ecological speciation is though still limited.

MHC - a magic trait driving ecological speciation

It is clear that immunogenes enabling rapid evolution of parasite resistance can play a major role in local adaptation and ecological speciation (Eizaguirre *et al.* 2009a). MHC genes are strong candidates for magic traits – they are highly diverse and specific, and show pleiotropic effects on natural and sexual selected traits. It has thus been proposed that MHC genes encode a magic trait involved in population differentiation and ultimately speciation (Eizaguirre *et al.* 2009a; Eizaguirre & Lenz 2010).

First, the specificity between MHC genotypes and parasites leads to locally adapted MHC genotypes (Sommer 2005; Piertney & Oliver 2006; Wegner 2008). Such associations result in habitat-specific MHC repertoires. There, contrasting immunogenetic allele pools provide the fuel for population differentiation and ecological speciation. MHC divergence between habitats has been shown mostly in fishes (e.g. Matthews *et al.* 2010; Eizaguirre *et al.* 2011; McCairns *et al.* 2011; Natsopoulou *et al.* 2012) but thanks to the development of genetic tools it is extending to other taxa (e.g. (Babik *et al.* 2008; Radwan *et al.* 2014). A recent long-term reciprocal transplant experiment revealed that MHC differences between these ecotypes indeed represented local adaptation to local parasites (Eizaguirre *et al.* 2012b). This experiment exposed laboratory-bred individuals to natural parasite communities, highlighting the importance of long periods of selection to identify patterns of local adaptation in semi-natural conditions.

Second, MHC is known to be involved in mate choice, hence facilitating local adaptation (Milinski 2006). Indeed, theory predicts that females will prefer males with specific MHC alleles or genotypes providing resistance against common local parasites. This selection for "good genes" can eventually lead to MHC-dependent assortative mating and to ecotype-specific mating preference (Milinski 2006; Eizaguirre *et al.* 2009a; b, 2011; Lenz *et al.* 2009b).

What are the conditions for parasites to drive reproductive isolation and cause speciation is still an open question. Although MHC-mediated mate choice is recognized throughout jawed vertebrates, the role of MHC-based mate preference as a reproductive barrier has only indirect support (but see (Blais *et al.* 2007; Eizaguirre & Lenz 2010; Raeymaekers *et al.* 2010; McCairns *et al.* 2011; Eizaguirre *et al.* 2011). Differences in parasite communities between populations do not necessarily lead to differences in MHC allele pools, even if MHC diversity and parasite load are correlated (Tobler *et al.* 2014, see Wegner 2008). In fact, balancing selection could then maintain genetic diversity at MHC loci while diversity on the rest of the

genome decreases, hence counteracting population differentiation and impeding the speciation process (Ricklefs 2010; Tobler *et al.* 2014). It results that, to understand the condition under which parasites promote or prevent speciation, it is necessary to investigate the strength of local parasite-mediated selection and the specificity of host-parasite interactions. Particularly, the role of MHC mediated natural selection against natural migrants is still to be determined.

1.2.4. Can gametic isolation play a role in ecological speciation?

Speciation often involves a combination of different reproductive barriers (Coyne & Orr. 2004). Post-mating pre-zygotic isolation includes processes that prevent fertilization after mating and the formation of hybrids (Howard 1999). These processes comprise limited transfer of viable sperm in heterospecific female tract, differential storage or use of heterospecific male sperm as well as incompatibilities between spermatozoa and eggs, preventing gamete binding or fusion (Coyne & Orr. 2004). These reproductive barriers can play an important role in population differentiation, particularly during reinforcement (Lorch & Servedio 2007; Lorch *et al.* 2011). There, genetic differentiation between isolated populations is expected to facilitate the evolution of further reproductive barriers (Servedio 2001; Ortiz-Barrientos *et al.* 2009). Indeed, post-mating barriers can contribute to reinforcement in concert with pre-mating barriers that are not absolute, particularly as occasional mating between ecotypes is possible. In cases of ecological speciation, natural and sexual isolation against migrants and hybrids represent commonly described fast-evolving barriers. However, the costs of producing unfit hybrids has the potential to promote pre-zygotic barriers limiting the production and development of hybrids (Ludlow & Magurran 2006; Immler *et al.* 2011).

Due to their cryptic nature, post-mating pre-zygotic barriers are challenging to investigate, hence the relatively low number of studies published to date (Eady 2001; Birkhead & Brillard 2007; Martín-Coello *et al.* 2009). The high level of species divergence in egg-sperm recognition proteins suggests gamete phenotypes play important roles in reproductive isolation (Turner & Hoekstra 2008). Sequence divergence in fertilization proteins is associated with assortative gamete preference at the species level. Genetic drift and ecological phenotypic divergence on gametic traits can therefore initiate and reinforce reproductive barriers during ecological speciation. The underlying mechanisms can involve neutral or adaptive divergence in molecules implicated in attraction and recognition between gametes or

in reproductive physiology (Palumbi 1994; Vacquier 1998; Swanson & Vacquier 2002; Geyer & Palumbi 2005).

Also between ecotypes, divergence in gamete and reproductive tract phenotypes might result in post-copulatory reproductive barriers. In the case of ecological speciation, divergent selection on sperm and egg characteristics due to differences in the environment (particularly in species with external fertilization) can theoretically lead to gametic isolation. Environmentally-mediated changes in sperm morphology have been suggested to contribute to differential fertilization success between species and thus to speciation (Immler et al. 2011). In addition to gamete divergence and gametic isolation *sensu strico*, local adaptation to parasite communities can result in reduced fertility as immune variation linked to parasite infection is expected to detrimentally affect sperm production and phenotype (Folstad & Karter 1992; Kurtz *et al.* 2007). As fertilization success can be related to infection and high infection rates, migrants would fertilize fewer eggs, leading to reduced gene flow (Liljedal *et al.* 1999; Kekäläinen *et al.* 2014).

One type of gametic barrier is conspecific sperm precedence (CSP) - preferential fertilization of eggs by sperm of conspecific males when occurs when females mate with both conspecific and heterospecific males (Servedio 2001; Coyne & Orr. 2004). CSP represents the gametic equivalent of assortative mating and conspecific mating preference across populations (or ecotypes). There, eggs from a local female are more likely to be fertilized by sperm from a local male than the sperm of a migrant male, when in competition. CSP seems to be a prevalent mechanism across species to reduce the production of hybrids (Hewitt *et al.* 1989; Wade *et al.* 1994; Price *et al.* 2000; Martín-Coello *et al.* 2009; Immler *et al.* 2011). CSP has also recently been shown between guppy populations separated for two million years (Ludlow & Magurran 2006) but not between recently isolated allopatric mice populations (Firman & Simmons 2014). Further investigation is therefore needed, particularly in the context of incipient ecological speciation.

1.3. Three spined stickleback: a model to study evolution in action

The three-spined stickleback (*Gasterosteus aculeatus* L.) is a small teleost fish, inhabiting marine, brackish and freshwater habitats in the Northern Hemisphere. Since Tinbergen in the mid-20th century (Tinbergen 1952), this established model system has provided much insight in many research fields such as spatial ecology (Milinski 1979), evolution of cooperation (Milinski 1987), sexual selection (Milinski & Bakker 1990; Reusch *et al.* 2001), host-parasite coevolution (Barber 2013) and speciation (McKinnon & Rundle 2002). Sticklebacks represent one of the rare vertebrate species which can be easily controlled in both laboratory and field experiments. The relatively short generation time allows following (parasite-mediated) selection at different life stages and conducting experiments across several generations to study trans-generational genetic and non-genetic effects (Figure 2).

Sticklebacks take at most one year to mature and usually undergo one breeding period during their lifespan. The male builds a nest out of plant material, attracts gravid females through a set sequence of behaviors and after the female lays her eggs in the nest, the male creeps trough the nest and spawns over the eggs (Wootton 1976). During sneaking events, another male can fertilize part of the clutch by opportunistically spawning before (or after) the nest owner spawns on the eggs (Wootton 1976). In this context, sticklebacks can experience sperm competition, therefore providing the theoretical basis for the evolution of sperm phenotypes (Gimenez-Bonafe 2000; Bakker *et al.* 2006; Elofsson *et al.* 2006; Pike *et al.* 2010) and associated outcomes of sperm competition (Zbinden *et al.* 2001).

Figure 2: The development of the three-spined stickleback: Parasite-mediated selection and reproductive barriers can occur at any developmental stage: gamete, zygote, juveniles or adults. From left to right : spermatozoa, developing eggs, juveniles from 3 days to 5 months post-fertilization, male and female stickleback and juveniles and red-throated adult male stickleback captured in the river. Picture credits: J. Kaufmann

Sticklebacks are ideal for studying sperm traits and sperm competition, as experimental *in vitro* fertilization is made convenient by external fertilization and because contrary to most teleosts, stickleback sperm does not require priming (Stockley *et al.* 1997; Elofsson *et al.* 2003). Computer Assisted Sperm Analysis (CASA) provides the opportunity to quickly and reliably estimate sperm concentration and motility (Kime *et al.* 1996, 2001). Ultimately such experiments can help to draw conclusions on post-copulatory mechanisms involved in transgenerational effects and reproductive barriers.

Freshwater populations of sticklebacks originated from marine colonization events following glacial retreat at the end of the Pleistocene (McKinnon & Rundle 2002). Most interestingly, the three-spined stickleback species complex includes replicated pairs of sympatric (and parapatric) divergent populations in diverse freshwater habitats (e.g. lake/streams; benthic/limnetic). These ecotypes show striking phenotypic differences, including feeding behavior, morphology and other life-history traits (e.g. Schluter 1995; Boughman *et al.* 2005; Berner *et al.* 2008; Hendry *et al.* 2009; Raeymaekers *et al.* 2010; Eizaguirre *et al.* 2011b). This provides the rare opportunity to study evolution in action during early stages of ecological speciation between diverging ecotypes. Recently, considerable advances have been made in identifying the phenotypes under divergent selection and the genetic bases for this phenotypic divergence between freshwater ecotypes (e.g. Berner *et al.* 2011; Roesti *et al.* 2014; Arnegard *et al.* 2014). Although parallel patterns of phenotypic divergence seems global across multiple ecotype pairs, genomic and transcriptomic analyses suggest very local selective pressures (but see Colosimo *et al.* 2005; Chain *et al.* 2014).

The system of lakes and rivers in northern Germany (i.e. my study populations) is intriguing for several reasons: (i) lake and river stickleback ecotypes can be found in independent drainage systems. (ii) these ecotypes show extremely limited gene flow, at both neutral and adaptive markers. (iii) lakes and rivers differ consistently in the composition of macroparasites fauna, with higher taxonomic diversity and parasite load in lakes (Reusch *et al.* 2001; Kalbe *et al.* 2002; Eizaguirre *et al.* 2011). Most probably as a result of parasite-mediated selection, the immune gene make-up in each ecotype is optimal to cope with the local parasite fauna. Divergent MHC allele pools and immunogenetic diversity between ecotypes provide specific defenses against local parasites (Kalbe & Kurtz 2006; Jäger et al. 2007; Eizaguirre et al. 2011, 2012a). In addition, ecotypes show habitat-specific innate immune responses at the transcriptomic and cellular level (Kalbe & Kurtz 2006; Scharsack *et al.* 2007; Lenz *et al.* 2013). Previous work has shown several reproductive barriers: by testing

female preference for conspecific males, Eizaguirre et al. (2011) found that lake and river females prefer conspecific males based on odors. In another experiment, Eizaguirre and colleagues (2012b) showed that local adaptation favored locally selected MHC alleles. These two mechanisms (i.e. assortative mating and local adaptation) can explain the low gene flow observed between lake and river populations. However insight is still lacking on other reproductive barriers which could play a major role prior to between-habitat encounters (i.e. migrant inviability) and act as post-copulatory reinforcement in a system where the costs of local maladaptation to parasites are high.

The toolbox available for this species makes three-spined sticklebacks a great model system to study parasite-mediated selection and speciation: Molecular tools allow the conduction of highly controlled experiments with the use of microsatellites markers in double-blind experiments or in identifying the outcome of sperm competition in an experimental context (Kalbe *et al.* 2009; Eizaguirre *et al.* 2009b; Lenz *et al.* 2009b). Moreover, high-throughput MHC genotyping trough Reference Strain Conformation Analysis (RSCA : Lenz *et al.* 2009a) allows rapid and reliable identification of MHC alleles and quantification of MHC diversity (Eizaguirre *et al.* 2009b; Lenz *et al.* 2009b).

Finally, a fascinating macroparasite taxonomic diversity can be found on and in three-spined sticklebacks (Figure 3, (Jakobsen 2011; Eizaguirre *et al.* 2011; Barber 2013). Moreover, the life cycles of some naturally-occurring parasites can be controlled in the laboratory and provide the rare opportunity of testing hypotheses of parasite mediated selection with experimental exposure in a vertebrate. Whereas most studies focus on one parasite or parasite taxa in the field, screening a wide range of macroparasites species allows for a more comprehensive understanding the complex selective pressures at play in parasite-mediated selection and parasite-mediated speciation.

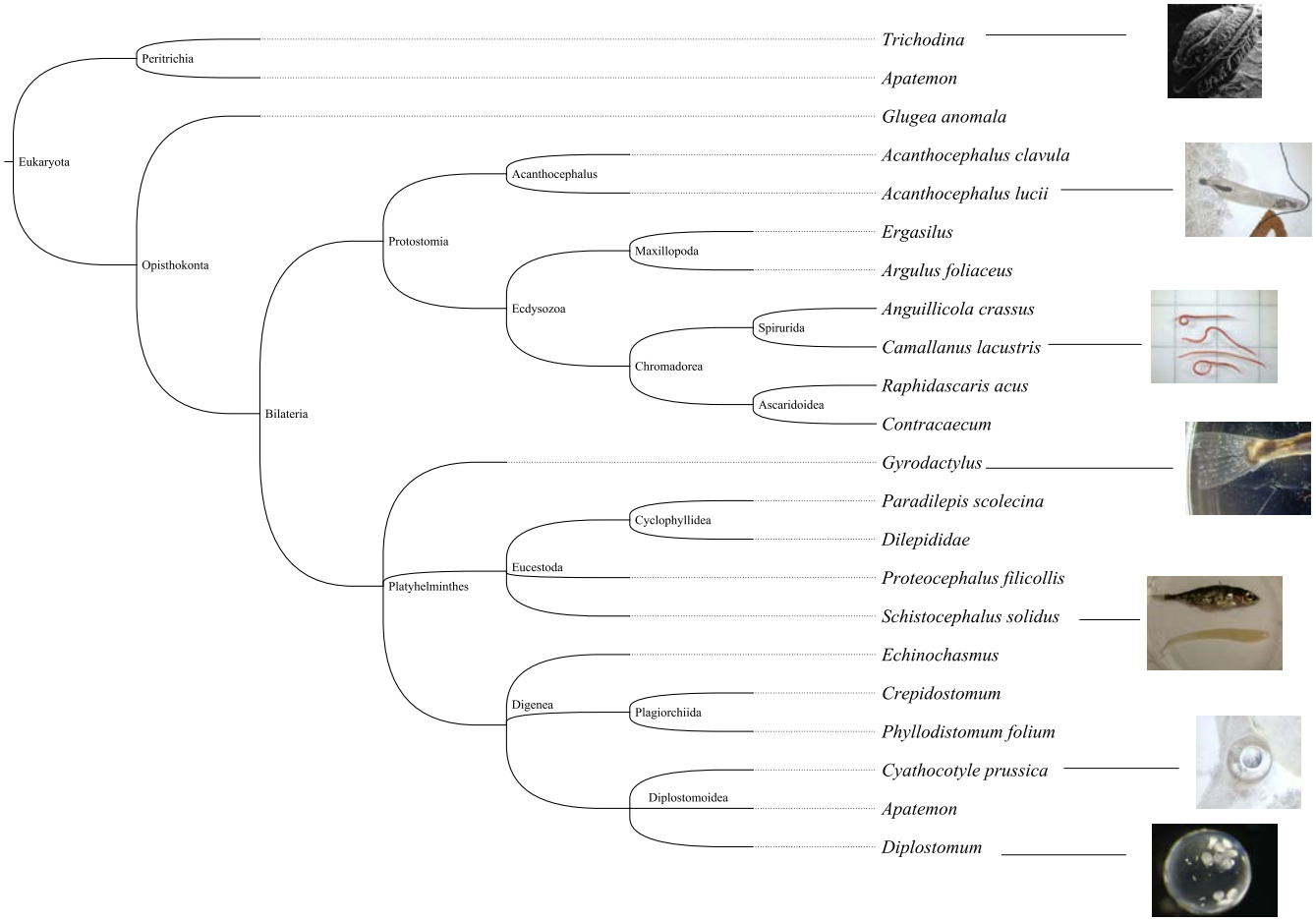


Figure 3: The great taxonomic diversity in macroparasites in three-spined sticklebacks: Species tree illustrating the diversity of macroparasites found in freshwater populations of three-spined sticklebacks. Many of these parasite taxa have been identified as major ecological selective pressure : The nematodes *Camallanus lacustris* and *Anguillicoloides crassus* have been shown to trigger rapid and adaptive evolution of MHC genes (Eizaguirre *et al.* 2012a). The tapeworm *Schistocephalus solidus* is developing as a model system for studying parasite local adaptation and the evolution of virulence (Heins & Baker 2008b; Henrich *et al.* 2013). Local adaptation to the eye fluke *Diplostomum sp.* has been shown to be mediated by innate and adaptive immune responses (Kalbe & Kurtz 2006; Scharsack & Kalbe 2014). The nodes show the higher taxa groups. Data from the NCBI Taxonomy database. Pictures credits: J. Kaufmann, except *Trichodina* (A.D.M. Dove), *Gyrodactylus* and *Diplostomum* (M. Kalbe).

Thesis outline

The aim of my doctoral work was to investigate the role of parasites in the evolution of host defenses and reproductive barriers. I first tested how ecology and specifically parasite-mediated selection could contribute to population differentiation and speciation in three-spined stickleback ecotypes. I then worked on assessing how paternal effects of parasite infection could evolve and be expressed. This thesis is organized in three chapters, presented in the form of independent manuscripts. These manuscripts represent three major projects of my PhD work and illustrate the development of my PhD research. Chapter I has been published in Biology Letters in 2015, Chapter III has been published in Ecology Letters in 2014 and chapter II will be submitted shortly. All projects have been conducted in cooperation with colleagues. The table provided at the end of this section shows a detailed overview of the authors' contribution.

Chapter I: The contribution of post-copulatory mechanisms to incipient ecological speciation in sticklebacks

In chapter I, I evaluated whether gametic isolation could evolve during early ecological speciation. Specifically, I tested for the first time the existence of assortative gamete preference between individuals from different ecotypes. Using replicated populations, I assessed ecotype-specific differences in sperm phenotypes between lake and river males and tested the outcome of *in vitro* sperm competition within and between individuals originating from lakes and rivers. Testing the relative paternity of sympatric males and evaluating developmental defects allowed a comprehensive evaluation of pre- and post-zygotic reproductive barriers (i.e. gamete preference and gametic incompatibilities).

Chapter II: Costs of migration in diverging three-spined sticklebacks and insights into the maintenance of immunogenetic polymorphism.

In the second chapter of my thesis, I tested for the existence of selection against migrants between lake and river stickleback ecotypes in this early phase of ecological speciation. Using a full-factorial field transplant experiment, I documented how migration affected macroparasite communities and condition- and immune- related traits (e.g. growth, spleen size). I also tested for associations between habitat-specific MHC haplotypes and the burden

of local and foreign parasites as it could explain the maintenance of immunogenetic diversity through fluctuating selection. By simulating dispersal between habitats, I could test for the role of local adaptation in maintaining low level of gene flow during early population differentiation. Particularly, using juveniles that already experienced parasite exposure in their native habitat prior to translocation permitted to test realistically for costs of migration in diverging three-spined sticklebacks.

Chapter III: Experimental parasite infection reveals costs and benefits of paternal effects

In the third chapter of my thesis, I examined the adaptive value of non-genetic transgenerational effects of infection. I first tested the consequences of infection on sperm phenotype and function using Computer Assisted Sperm Analysis and sperm competition trials. With a split-clutch design controlling for genetic variation and maternal effects, I could also evaluate costs and benefits in the offspring upon infection. By using *in vitro* fertilization and managing to limit the effect of other contributors to variation in offspring phenotype, these experiments pinpointed the influence of paternal effects in shaping offspring life history, resistance and tolerance against parasites.

Table of contributions:

	Chapter I	Chapter II	Chapter III
Conception and design	CE, **JK**, TLL	CE, **JK**, TLL, MM	CE, **JK**, TLL, MM
Conducted the research	**JK**	CE, MK**, JK**	**JK**
Data analysis	**JK**	**JK**	**JK**
Interpretation and Writing	CE, **JK**, TLL, MM	CE, **JK**, TLL	CE, **JK**, TLL, MM

Authors are given in alphabetical order: CE: Christophe Eizaguirre; MK: Martin Kalbe; JK: Joshka Kaufmann; TLL: Tobias L. Lenz; MM: Manfred Milinski

Chapter I

The contribution of post-copulatory mechanisms to incipient ecological speciation in sticklebacks

Published as:

Joshka Kaufmann[1], Christophe Eizaguirre[2,3], Manfred Milinski[1], Tobias L. Lenz[1] (2015). The contribution of post-copulatory mechanisms to incipient ecological speciation in sticklebacks. *Biol. Lett.*, in press (2015)

Short title: Post-copulatory mechanisms in speciation

Keywords: ecological speciation; gamete precedence; *Gasterosteus aculeatus*; fertilization success; ecotype

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.68t78

Summary

Ecology can play a major role in species diversification. As individuals are adapting to contrasting habitats, reproductive barriers may evolve at multiple levels. While pre-mating barriers have been extensively studied, the evolution of post-mating reproductive isolation during early stages of ecological speciation remains poorly understood. In diverging three-spined stickleback ecotypes from two lakes and two rivers, we observed differences in sperm traits between lake and river males. Interestingly, these differences did not translate into ecotype-specific gamete precedence for sympatric males in competitive *in vitro* fertilization experiments, potentially due to antagonistic compensatory effects. However, we observed indirect evidence for impeded development of inter-ecotype zygotes, possibly suggesting an early stage of genetic incompatibility between ecotypes. Our results show that prezygotic post-copulatory mechanisms play a minor role during this first stage of ecotype divergence, but suggest that genetic incompatibilities may arise at early stages of ecological speciation.

Introduction

Studying recently diverged species has provided important insights into the processes and mechanisms of speciation (Coyne & Orr. 2004). Ecological speciation defines the evolution of reproductive barriers caused by divergent natural selection in different ecological niches or habitat types (Rundle & Nosil 2005), leading to the formation of ecological demes (ecotypes). Distinct mechanisms can generate and maintain reproductive isolation between ecotypes such as local adaptation, selection against hybrids or preference for conspecific mates (Rundle & Nosil 2005; Maan & Seehausen 2011; Eizaguirre *et al.* 2012b). Surprisingly, to date only a limited number of studies have examined the importance of post-copulatory processes in the context of incipient ecological speciation even though post-copulatory traits can initiate, reinforce and maintain reproductive isolation (Eady 2001; Lorch & Servedio 2007). Under divergent ecological selection regimes, males may for instance evolve different sperm characteristics (i.e. morphology, velocity) which may facilitate conspecific female fertilization or increase sperm competitiveness in the local environment (Elofsson *et al.* 2006). Furthermore, gametic incompatibilities could occur, based on allelic or genotypic differences, between partners from different populations (Eady 2001).

Three-spined sticklebacks (*Gasterosteus aculeatus spp.*, L.) have become a model organism in ecological speciation (McKinnon & Rundle 2002). Ecological contrasts between habitats have led to divergent selection on various traits such as morphology, feeding behavior and mating systems (Boughman *et al.* 2005; Raeymaekers *et al.* 2010; Maan & Seehausen 2011). Sticklebacks are external fertilizers that experience sperm competition in the context of alternative male mating strategies, such as sneaking, and thus facilitate the investigation of post-copulatory reproductive barriers (Wootton 1976). Particularly, externally ejaculated spermatozoa are directly confronted with habitat-specific ecological conditions during spawning which provides the potential to evolve different sperm traits in contrasting habitats (Elofsson *et al.* 2006).

The system of lakes and rivers in northern Germany is a young post-glacial system where different stickleback ecotypes can be found (Reusch *et al.* 2001). In this system, local adaptation (Eizaguirre *et al.* 2012b) and female preference for sympatric males (Eizaguirre *et al.* 2011) contribute to pre-copulatory reproductive barriers. Here, we i) evaluate sperm traits in replicated populations and ii) experimentally test for ecotype-specific gamete precedence between lake and river stickleback ecotypes.

Material and methods

Study system

Three-spined sticklebacks were caught from two independent pairs of geographically connected lake and river populations, representing two drainage systems in northern Germany (Electronic supplementary material S1). After 20 weeks under standardized winter-like conditions (8h day:16h night; 6°C) and six weeks in spring-like conditions (12h:12h; 12°C), fish were isolated singly into 16 L tanks under summer conditions (16h:8h; 18°C). There, to build a nest, males were provided with artificial nesting material (Sommerfeld *et al.* 2008).

Experimental design

We performed *in vitro* sperm competition trials following a full-factorial design involving a female, a sympatric male, and a 'competing' male. This competing male was either from the same ecotype as the female or from a different ecotype. Three to six such triads were conducted for each of the 16 possible population combinations (Electronic supplementary material S2). This resulted in 64 independent sperm competition trials (33 within-ecotype and 31 between-ecotype). In trials with two sympatric males, we randomly declared one male as the focal male. Individuals were used once only.

Sperm measurements and competition trials

All males were presented with a ripe female within 24 hours prior to dissection and showed active reproductive behavior, e.g. nest gluing (Sommerfeld *et al.* 2008). Fish were sacrificed by an incision in the brainstem prior to dissection. After dissection, sperm was isolated by mashing the entire testes in 900 µl of HBSS solution. We measured sperm concentration as well as curvilinear, straight-line and average-path velocities using computer assisted sperm analysis (Electronic supplementary material Methods). Each egg clutch was carefully stripped into a dry Petri dish and fertilized by a mixture containing 20 µl of sperm solution from each male in 5 ml of fresh water. Differences in ejaculate traits thus reflect natural conditions. Fertilized eggs were reared under controlled conditions with oxygenated water. Five days after fertilization, eggs were counted and categorized into unfertilized (no visible zygote), undeveloped (dead zygote) and developed eggs (Swarup 1958). An insufficient number of fertilized eggs in seven clutches (four within-ecotype, three between-ecotype) led to a total of

57 independent trials. We genotyped all fertilized eggs ($N_{total\ eggs}$=2508) and parents (N_{adults}=171) at 5 microsatellite loci and identified the most likely sire using CERVUS v3.0.3 (Field Genetics Ltd; (Kalinowski *et al.* 2007); Electronic supplementary material Methods).

Statistical analyses

Relative paternity and the proportion of undeveloped eggs were normalized using arc-sinus and log transformation, respectively. First, we analyzed differences in sperm traits (concentration and sperm velocity) between ecotypes using ANCOVAs with ecotype, drainage system and their interaction as cofactors and testes mass as covariate (lm function in R). Second, we performed an analysis of covariance (lm function in R) on the proportion of eggs sired by the sympatric male (relative paternity). The full model included female ecotype and the type of competition (within-ecotype vs. between-ecotype) as factors, and relative spermatozoa concentration, relative velocity (sympatric/competing) and their two-way interaction as co-variables. Due to multi-colinearity, we corrected the explanatory co-variables using the residuals of sperm concentration on testes mass and ecotype identity and the residuals of sperm velocity (PC1 values, Electronic supplementary material Methods) on testes mass, total sperm concentration and ecotype identity. The best fitting model was selected using an AIC-based backward selection procedure (stepAIC function in R). We similarly tested for variation in the proportion of undeveloped eggs in relation to paternity and the type of competition. All statistical tests were conducted in R v. 3.0.3 (R Development Core Team 2014).

Results

We found that sperm velocity was significantly higher for lake males than river males ($F_{1,141}$=9.07, p=0.003, Electronic supplementary material S3, Fig. 1a) while river fish showed higher sperm concentration ($F_{1,141}$=9.52, p=0.002, Electronic supplementary material S3, Fig.1b). These differences suggest ecotype-specific sperm characteristics even though their extent varied between the population pairs as indicated by significant interactions between ecotype and drainage system (Electronic supplementary material S3).

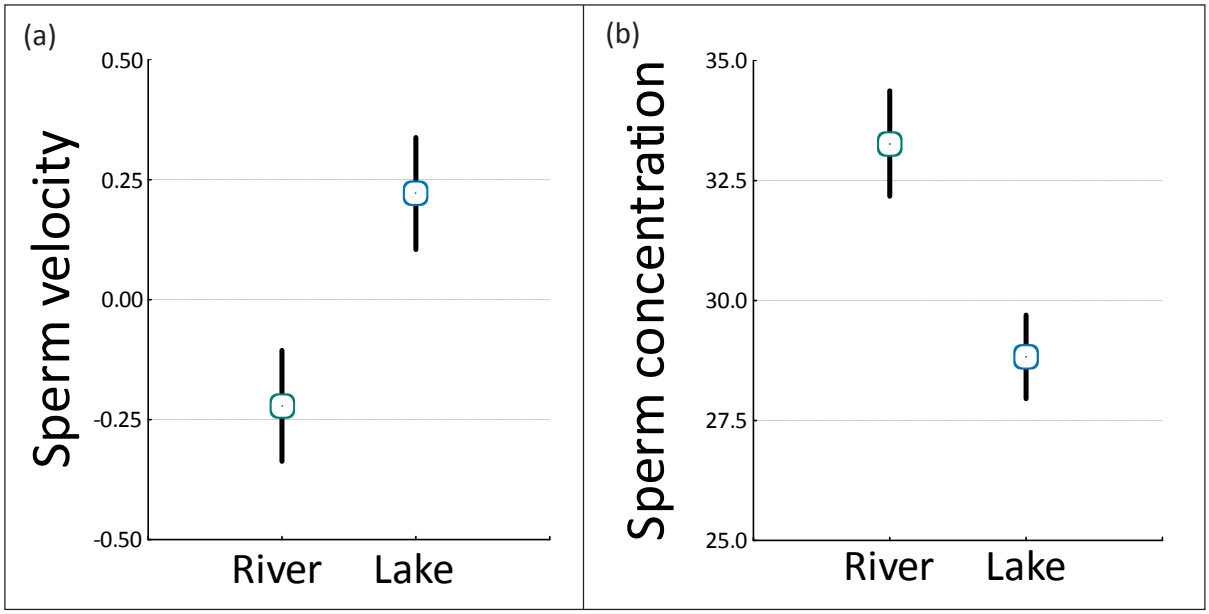


Figure 1: Shown are means (± 1 S.E.M.) of (a) sperm velocity (standardized residuals) and (b) total spermatozoa concentration (in 1.5 µL/mg of testes) of river and lake males.

These sperm characteristics did not translate into significant sperm precedence for sympatric males ($F_{1,57}$=0.11, p=0.744, Table 1). Our sample size provided sufficient statistical power to detect sperm precedence of magnitudes similar to those reported in other species pairs (h=0.835, power>0.99, Electronic supplementary material S4). Both relative velocity and relative spermatozoa concentration between competing males were strong predictors of paternity (velocity: $F_{1,57}$=10.91, p=0.002; concentration: $F_{1,57}$=10.12, p=0.003).

Interestingly, we found that the origin of the competing male significantly affected the proportion of undeveloped eggs in interaction with the relative paternity of the sympatric male

($F_{2,57}$ =4.160, p=0.021): the relationship between paternity and the proportion of undeveloped eggs was significant only when sperm competition involved males from different ecotypes (between-ecotype: r=0.442, 95%CI=0.09-0.69, p=0.016; within-ecotype: r=0.183, 95%CI=-0.18-0.51, p=0.32, Fig. 2).

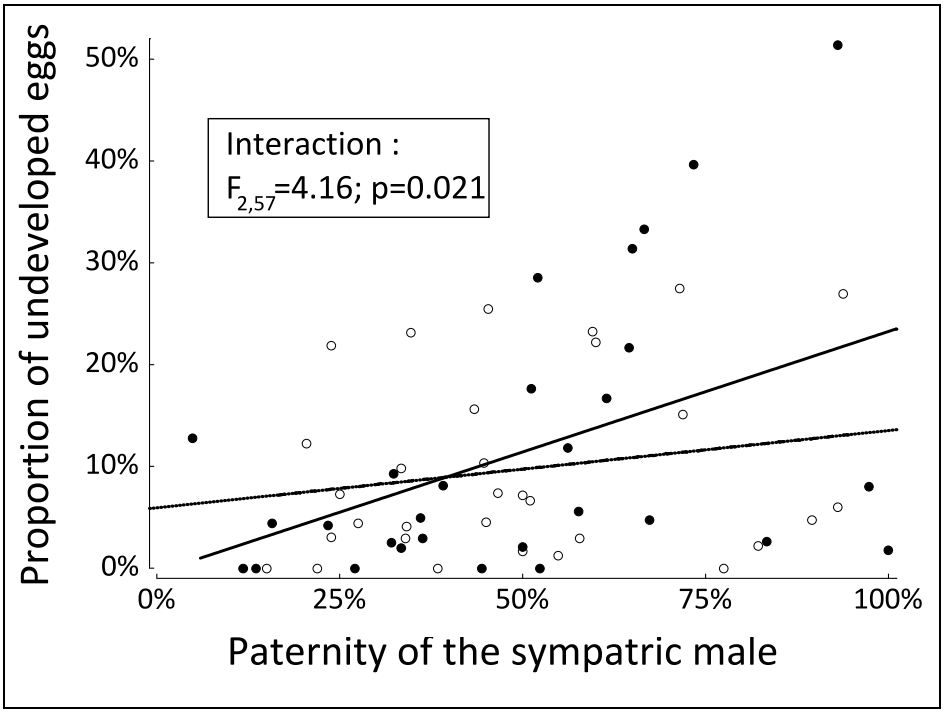


Figure 2: Relationship between paternity of the sympatric male and the proportion of undeveloped eggs. Filled circles and solid regression line (significant) correspond to between-ecotype trials and open circles and dashed line (non-significant) to within-ecotype trials.

Table 1: Effects of sperm characteristics and ecotype origin on (a) paternity of sympatric male and (b) proportion of undeveloped eggs. Statistical table showing results of linear models. Only variables included in the best fitted model are shown. (d.f.: degrees of freedom, S.S.: sum of squares). Significant effects are printed in bold.

(a) Paternity of sympatric male				
	d.f.	S.S.	F-value	p-value
Relative sperm speed	**1**	**0.771**	**10.91**	**0.002**
Relative sperm concentration	**1**	**0.715**	**10.12**	**0.003**
Origin of the competing male	1	0.008	0.11	0.744
Proportion of undeveloped eggs	**1**	**0.605**	**8.56**	**0.005**
Origin of competing male : Relative sperm concentration	**1**	**0.329**	**4.66**	**0.035**
Residuals	52	3.673		
(b) Proportion of undeveloped eggs				
	d.f.	S.S.	F-value	p-value
Paternity of sympatric male : origin of the competing male	**2**	**0.071**	**4.16**	**0.021**
Residuals	57	0.484		

Discussion

Investigating post-copulatory reproductive isolation between three-spined stickleback ecotypes, we found differences in sperm traits between males from lake and river. Particularly, river males showed higher sperm concentration and slower spermatozoa speed than lake males. Differences in these sperm traits may stem either from neutral processes or from contrasting abiotic and biotic ecological pressures between habitats known to affect such phenotypes (e.g. temperature (Breckels & Neff 2014), parasites (Kaufmann *et al.* 2014)). However, despite variation in the extent of sperm trait differentiation between two independent drainage systems, the fact that we found a parallel pattern suggests an ecological origin of these differences.

Our experiment confirmed that sperm number and sperm velocity are major predictors of paternity in fishes, particularly during sperm competition (Stockley *et al.* 1997). Although sperm traits diverged between ecotypes, these differences did not lead to biased paternity for sympatric males in between-ecotype competition trials and thus did not translate into post-copulatory pre-zygotic reproductive isolation. We can hypothesize that high sperm concentration in lake males and high sperm speed in river males might act in a compensatory manner in competitive situations, leading to balanced paternity. Also, pre-copulatory reproductive isolation is relatively strong in this system, which may decrease selection for sympatric sperm precedence as a reproductive barrier (Eady 2001; Eizaguirre *et al.* 2011).

Interestingly, however, we found a positive correlation between the proportion of undeveloped eggs and paternity of the sympatric male in clutches where males from different ecotypes competed for fertilization. In other words, with more eggs failing to develop, the remaining developing eggs were more likely to have been sired by the sympatric male. The fact that this correlation could not be observed in competition trials between ecologically-equivalent (same ecotype) males suggests that the developmental failure could be due to emerging Dobzhansky-Muller-type genetic incompatibilities between the diverging stickleback ecotypes (Stelkens *et al.* 2010; Crespi & Nosil 2013). Such reproductive barriers at the zygote stage have been shown in many taxa, but so far mostly between distinct species with no gene flow (Rundle 2002; Immler *et al.* 2011). Unfortunately, genotyping of the undeveloped eggs, which would be necessary to confirm the above hypothesis, was impossible due to the low DNA concentration and quality. Therefore, further investigation is required to confirm this observation.

Altogether, our results show that reproductive isolation in response to ecological adaptation does not necessarily arise at all levels simultaneously. The suggested genetic incompatibility in turn may have arisen as a by-product of reduced gene flow, driven by forces such as mate choice and local adaptation, in an otherwise open system (Lorch & Servedio 2007). If confirmed by further experimental effort, this would suggest a role for genetic incompatibilities in reproductive isolation between contrasting ecological habitats already at an early stage of speciation.

Acknowledgments

We thank G. Augustin, D. Martens, S. Bartel, G. Schmiedeskamp and I. Schultz for technical assistance. M. Kalbe, D. Benesh, and two reviewers provided helpful comments.

Ethics statement: All experiments were approved by the Ministry of Energy, Agriculture, the Environment and Rural Areas, Schleswig-Holstein, Germany.

Chapter II

Costs of migration in diverging three-spined sticklebacks and insights into the maintenance of immunogenetic polymorphism

Authors: Joshka Kaufmann[1], Tobias L. Lenz[1], Martin Kalbe[1], Manfred Milinski[1], Christophe Eizaguirre[2,3]

[1]Max Planck Institute for Evolutionary Biology, Department of Evolutionary Ecology, Plön, Germany

[2] GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany

[3] School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom

Abstract

Local adaptation is often key to the process of speciation. Theory suggests that reduced fitness of migrants due to local maladaptation could be sufficient to reduce gene flow and ultimately lead to speciation. Here, we experimentally investigated the relative fitness of migrants in foreign habitats, focusing on diverging lake and river ecotypes of three-spined sticklebacks. A reciprocal transplant experiment performed in the field revealed asymmetric costs of migration: while mortality of river migrants was increased under lake conditions, lake migrants suffered from reduced growth relative to river residents. Focusing particularly on the parasitic environments, we found that macroparasite communities did not only differ between lake and river residents but also between the reciprocal migrants. This pattern of differential parasitisation had consequences for both the innate and the adaptive immune system, where multiple habitat-specific associations between parasite species and locally selected alleles of major histocompatibility immunogenes could be detected. Altogether, these experimental results highlight the role of selection against migrants in the early stages of ecological speciation and reveal complex resistance patterns leading to immunogenetic diversity at the meta-population level.

Introduction

Understanding the mechanisms contributing to reproductive isolation during the onset of speciation is a fundamental question in evolutionary biology (Coyne & Orr. 2004). It is now accepted that ecological factors play a major role in the process of population differentiation, reproductive isolation and ultimately speciation (Rundle & Nosil 2005; de León *et al.* 2010; Rosenblum & Harmon 2011). Throughout ecological speciation, reproductive barriers can evolve between parapatric or sympatric populations due to adaptive divergence to different habitats (Schluter 2000; McKinnon *et al.* 2004). Although both pre- and post-copulatory processes have now been reported, the role of ecology in the formation and maintenance of reproductive barriers is still incompletely understood (Boughman 2001; Coyne & Orr. 2004; Maan & Seehausen 2011). Particularly, local adaptation to ecological conditions acting and the potentially resulting maladaptation of migrants (and hybrids) is a powerful process limiting gene flow between sympatric or parapatric populations, providing high ecological selective pressure (Hendry 2004; Nosil *et al.* 2005; Eizaguirre *et al.* 2012b; Peterson *et al.* 2014). Costs limiting gene flow between locally adapted demes (i.e. ecotypes) can be manifold and include reduced survival, body condition but also suffering from inadequate gene regulation (Nagy & Rice 1997; Hendry 2004; Eizaguirre *et al.* 2012b; Lenz *et al.* 2013).

Among the variety of environmental variables, parasites are an ubiquitous, diverse and strong ecological selective pressure, affecting immune characteristics, host condition, and ultimately Darwinian fitness (Hamilton & Zuk 1982; Lively 1999; Poulin 2006; Kalbe *et al.* 2009). As such, they can act against locally maladapted migrants and ultimately reinforce speciation between ecologically contrasting habitats. Under local adaptation, hosts can become better adapted to local parasites, providing the selection parasites exert is spatially constrained and host gene flow limited (Kawecki 1998; Lajeunesse & Forbes 2002; Kalbe & Kurtz 2006). Here we will define local adaptation from the host point of view, where hosts ultimately perform better in their own environment compared to a foreign environment (Kawecki & Ebert 2004). Differences in parasite communities due to abiotic or biotic factors between habitats are likely to arise (MacDougall-Shackleton *et al.* 2002; Eizaguirre *et al.* 2011, 2012b; Raeymaekers *et al.* 2013; Lenz *et al.* 2013). Ultimately, local adaptation is expected to induce high costs on migrants (Kawecki 1998; Eizaguirre *et al.* 2012b). Local adaptation to different parasite species and communities is mostly mediated by host immunocompetence, via

inherited differences in both innate and adaptive immune defenses (Ebert & Hamilton 1996; Poulin *et al.* 2011).

As part of the specific adaptive immune system acting against macroparasites, the major histocompatibility complex class II (MHC II) has the potential for a strong role in local adaptation (Eizaguirre *et al.* 2009a, 2011, 2012b; Eizaguirre & Lenz 2010). MHC class II genes code for surface molecules, which present antigens derived from extracellular pathogens to T-cells. This leads to the activation of adaptive immunity (e.g. immune cell proliferation, antibody production). This specific association and the unparalleled diversity at the MHC explain why so far almost no other genes have shown similar signatures (but see Tschirren *et al.* 2013 for other immune genes). MHC allele pools diverge rapidly between populations from habitats with contrasting parasite communities most likely as a result of associations between habitat-specific MHC alleles and parasite species (birds: Loiseau 2011; voles: Tollenaere et al. 2008; salmonids: Landry and Bernatchez 2001, Dionne et al. 2009; newts: Babik et al. 2008). Parasite-mediated selection can lead to immunogenetic local adaptation where hosts carrying maladapted MHC genotypes in a foreign habitat will suffer from increased susceptibility to local parasites (Summers *et al.* 2003; Blais *et al.* 2007; Eizaguirre *et al.* 2009a, 2011, 2012b; Eizaguirre & Lenz 2010).

Similarly, habitat-specific differences in the expression of innate immune genes upon parasite exposure show that those genes can also contribute to the evolution of local adaptation (Lenz *et al.* 2013). It is therefore important to investigate both adaptive and innate immune parameters when looking at potential parasite-related costs in an ecological context. The activation of the immune system can be estimated by examining the phenotypes of immune cell proliferation (granulocytes representing mainly the innate immune system and lymphocytes representing mainly the adaptive system) and of spleen weight - which significantly increases upon infection (Lefebvre & Mounaix 2004; Kalbe & Kurtz 2006). To date, only a small number of experimental studies involving exposure to natural parasite communities have shown a disadvantage of migrants facing foreign parasites (MacColl & Chapman 2010; Eizaguirre *et al.* 2012b). This is likely due to the challenge of simulating migration under controlled conditions, while allowing for natural selection by parasites to take place. The use of reciprocal transplant experiments *in natura* overcomes the shortcut of cultivating a low number of parasites in the and also allows to consider parasite communities in their natural environment (Rauch *et al.* 2006; Eizaguirre *et al.* 2012b).

Three-spined sticklebacks (*Gasterosteus aculeatus* L.) represent an ideal study system to experimentally test for costs of migration in relation to parasite infections and immunogenetic diversity (Barber 2013). Inhabiting marine as well as diverse freshwater habitats across the Northern hemisphere, sticklebacks are recognized for their rapid adaptive potential to colonize contrasting habitats (reviewed in (McKinnon & Rundle 2002). Recent studies have shown the importance of local adaptation to contrasting parasite communities in the adaptive differentiation between stickleback populations (Kalbe & Kurtz 2006; MacColl 2009; Eizaguirre *et al.* 2012b; Konijnendijk *et al.* 2013; Feulner *et al.* 2015). Sticklebacks from lakes and rivers in Northern Germany show very limited gene flow, although both migration in nature and interbreeding in the laboratory are possible (Reusch et al. 2001; Kalbe & Kurtz 2006, Eizaguirre et al. 2011). In this system, MHC allele pools differ between populations from different habitats and there exists costs on hybrids (Eizaguirre *et al.* 2011, 2012b). In addition, habitat-specific adaptation of the innate immune system might play a significant role in selection against migrants as individuals can evolve higher levels of innate immune responses when challenged with a relatively more diverse and virulent parasite community (Kalbe & Kurtz 2006; Scharsack *et al.* 2007; Lenz *et al.* 2013; Scharsack & Kalbe 2014).

In this study, we specifically focus on the possible costs of migration between lake and river habitats in fish survival, growth and parasite infection using a reciprocal common garden field experiment (Table 1). This design further enabled us to evaluate the impact of simulated dispersal on innate and adaptive immunity (Eizaguirre et al., 2012a; Tollenaere et al., 2008). Given the system and following the theory of local adaptation, we predict a local competitive advantage for individuals (and genotypes) in their own environment (i.e. residents) against individuals immigrating from a foreign environment (i.e. migrants) (Kawecki & Ebert 2004). To reveal the underlying mechanisms of parasite-mediate selection against migrants, we explicitly investigated the relative role of the innate and the adaptive immune responses.

Table 1: Experimental design of the common garden field experiment, showing the used terminology and the number of fish used per treatment.

		Habitat of exposure	
		Lake	River
Ecotype of origin	Lake	Lake resident (n=50)	Lake migrant (n=50)
	River	Lake migrant (n=50)	River resident (n=50)

Materials and Methods

Fish collection

Young-of-the-year three-spined sticklebacks were collected in October 2009 from the "Grosser Plöner See" lake (54°9'21.16" N, 10°25'50.14" E, Germany) and the "Malenter Au" river (54°12'15.08" N, 10°33'41.90" E, Germany) using minnow traps and hand nets. The two sites are ca. 12 kilometers apart and belong to the same drainage system (Reusch *et al.* 2001; Eizaguirre *et al.* 2011). After capture, individuals were kept in the laboratory in 190 L aquarium per population with constant freshwater and oxygen supply under standardized winter conditions (8 h. day: 16 h. night; 6°C). Individuals were weighed (± 0.1 mg), measured (± 1 mm) and a spine was clipped for DNA fingerprinting and sex identification using sex-linked genetic markers (Griffiths 2000).

Experimental set-up

The fish were transferred to experimental mesocosms (1 m x 0.25 m x 0.6 m; length x height x width) in March 2010 at the original sampling locations (Eizaguirre *et al.* 2012b). We placed five mesocosms in each habitat for a total of 200 experimental fishes (100 per habitat). Each mesocosm contained 10 randomly chosen individuals (5 males and 5 females) from each ecotype (i.e. N=20 per mesocosm). Mesocosms were positioned at intervals of 10m and at an

approximate depth of 1 meter in the middle of the river and similarly along the shoreline in the lake. The 5mm mesh of the mesocosm allowed food items and intermediate hosts of various parasite species to pass through, while preventing fish from escaping. In addition, another partition inside the mesocosms separated males and females in order to prevent mating. Weekly and during 15 weeks, each mesocosm was inspected for dead fish, which were removed and sampled for later DNA fingerprinting. At the end of the experimental period we dissected all remaining live fish (n=133, July 2010). Forty fish (10 from 4 different mesocosms) were collected and dissected each day.

Dissections

Fish were measured, weighed and euthanized by an overdose of tricaine methane sulphonate (MS-222; 1.5 g/L). We calculated the splenosomatic index (relative spleen mass, weighed to the nearest 0.01 mg) to estimate immune response to parasite infections (Lefebvre and Mounaix 2004). We also calculated an individual condition factor based on the residuals of the log-log regression between total mass and standard length. Each individual was systematically screened for presence and number of macroparasites. To this end, fish were inspected for both external and internal parasites on the skin, fins, gills, eyes, body kidney, liver, gut, urinary bladder, swim bladder, gall bladder and muscles. Details on parasite screening can be found in Kalbe et al. (2002). The number of parasite taxa per individual (S), the Shannon diversity index (H'), (Clarke 1993; Kalbe *et al.* 2002) and an individual parasite index combining species-specific parasite load and community diversity (Kalbe *et al.* 2002) were used to describe parasite infection.

Immunological measurements

We performed flow cytometric analyses of head kidney leucocytes following protocols developed for three-spined sticklebacks described in Scharsack et al. (2004). In short, after isolating head kidney leucocytes in a cell suspension, the number of live granulocytes and lymphocytes were counted using a flow cytometer (FACSCalibur, Becton and Dickinson, USA) and the CELLQUEST PRO v. 4.02 software. This allowed for the calculation of individual granulocytes to lymphocytes (G/L) ratios, where, for example, a relative lower G/L ratio signifies higher levels of activation of the adaptive immune system in relation to the innate immune system.

DNA fingerprinting

Genomic DNA from spine samples (from initial measurements) and caudal fin samples (from dead and dissected fish) was extracted using the DNAeasy Blood and Tissue kit (Qiagen, Sussex, UK) following the manufacturer's protocol. All samples were genotyped for twelve polymorphic microsatellite loci combined into three multiplex PCR protocols (see Kalbe et al. 2009) to identify the fish at the end of the experiment. Furthermore we calculated a specific growth rate (SGR=100*ln(final mass)/ln(initial mass)) corrected for mass at the beginning of the experiment (following Scharsack et al. 2007).

MHC IIB genotyping

To identify individual MHC class IIB genotypes, we used reference strand-mediated conformation analysis (RSCA) optimized for sticklebacks as described in (Lenz *et al.* 2009a). We amplified the exon 2 of the MHC IIB gene, which encodes for the peptide-binding region of the MHC molecule. Duplication of MHC loci in the *G. aculeateus* genome leads to strong linkage disequilibrium, creating sequence variant combinations segregating in stable haplotype blocks (Reusch *et al.* 2004; Lenz *et al.* 2009a). We therefore later refer to MHC variants as MHC haplotypes. This MHC genotyping protocol has been used extensively in these populations (Lenz *et al.* 2009b; Eizaguirre *et al.* 2011, 2012a), allowing us to reliably obtain haplotype identity and sequence information for each individual. Also, higher levels of MHC diversity in term of allele numbers or higher intra-individual allelic divergence can be selected to provide resistance to a more diverse parasite community (Wegner *et al.* 2003b; Milinski 2006; Lenz 2011; Eizaguirre *et al.* 2012b).We used allele number and the mean MHC genetic distance within an individual as measures of MHC diversity. The latter was calculated as the average pair-wise amino acid p-distance between all sequence variants within an individual (see Lenz *et al.* 2009b).

Statistical analyses

Statistical analyses were conducted using R statistical package (R Development Core Team 2014). Normality and variance homoscedasticity of model residuals were verified and tests were conducted accordingly. Splenosomatic index and GL ratio were log-transformed to fit a normal distribution of residuals.

a) Mortality

We assessed mortality between experimental groups using a generalized linear mixed effect model with mortality as dependent variable (coded as 0: live and 1: dead), migration treatment (Resident vs. Migrant), habitat of exposure (lake vs. river), sex and initial mass as fixed predictors. The replicated mesocosms were set as a random factor. The significance of each variable as well as the interaction between habitat of exposure and migration treatment was tested with type II Chi-square based likelihood-ratio tests (based on a binomial distribution with logit function; glmer and Anova functions in R). To test differences in mortality rates within each habitat, we additionally performed Pearson's Chi-square tests with a p-value based on a Monte Carlo simulation (n=9999).

b) Effects of migration on fish condition and immune system

We tested for the effect of migration on growth, body condition and immune relevant traits (splenosomatic index and G/L ratio) using linear mixed effect models. The fixed predictors were migration treatment, habitat of exposure, sex and individual parasite load. Standard length was included only in the models on immune traits as calculations of growth and body condition already include individual standard length. Experimental mesocosm identity was set as a random factor. The 2^{nd} and 3^{rd} order interactions including migration treatment, habitat, and parasite load were implemented in the models as we were particularly interested in trait differences between migrants and residents in each habitat and the role of parasite load in these phenotypic differences. As the aim of the study was to focus on fitness-related traits between residents and migrants within each habitat, the effect of ecotype of origin is not included but confounded in the interaction between habitat of exposure and migration treatment. However, the pairwise differences tested with Tukey's honest significant differences method provide us with informative quantitative differences and allows us to disentangle within-habitat effect from overall differences based on the ecotype of origin.

c) Differences in parasite communities between residents and migrants

To test whether parasite communities differed between migrant and resident individuals within and between habitats, we used a multivariate permutation analysis (Permanova, adonis function in R) on a Bray–Curtis dissimilarity matrix based on log transformed parasite abundance. This nonparametric MANOVA allows partitioning the variation in distance matrices among multiple variables. The model included habitat of exposure and migration treatment as fixed predictors and experimental mesocosm as a random factor. We identified the parasites contributing most to the difference between treatments using a similarity percentage test (simper function in R). We tested for the effect of simulated migration on individual parasite diversity (Shannon index) and parasite load using linear mixed effect models with migration treatment, habitat of exposure and MHC genetic distance (or individual allele number) as fixed predictors and experimental mesocosm as random factor.

d) Linking MHC and macroparasite infection

Similarly we investigated whether MHC IIB haplotype pools differed between individuals of lake and river origin using an analysis of similarity (anosim function in R) on a Jaccard dissimilarity matrix based on the presence/absence matrix regrouping all haplotypes for all individuals (Gower & Legendre 1986). We identified the haplotypes contributing most to the difference between lake and river ecotypes using a similarity percentage test (simper function in R), allowing us to specifically single out haplotypes involved in local adaptation (Eizaguirre *et al.* 2011, 2012a). We examined whether selection at MHC IIB genes occurred by testing differences in MHC allele number, allelic divergence as well as in the MHC haplotype pool between dead and live fish in each habitat. We used permutation analyses on the MHC dissimilarity matrix with migration treatment and mortality (alive vs. dead) as fixed predictors and experimental mesocosm as a random factor. In order to test whether MHC diversity and parasite diversity were associated, we used a Mantel test with 9999 permutations correlating two distance matrices based on MHC IIB haplotype matrix and the parasite abundance community matrix (mantel function in R). Mesocosm was included as a random factor. Finally, to test for specific patterns of MHC-dependent resistance or susceptibility in a given habitat of exposure, we used generalized linear models with the number of parasites in infected fish as dependent variable (based on a Poisson distribution with log function), the absence/presence of the most common and divergent haplotypes (identified with the similarity analysis) and the habitat of exposure as fixed predictors. Due to non-independent testing, we

adjusted p-values for multiple comparisons using the false discovery rate (p.adjust function in R). Nine non-independent tests were performed: three parasites were each tested in association with three haplotypes. We report the outcome of each statistical model with type II ANOVAs with Kenward-Roger correction for F-statistics and d.f. in linear mixed models (lmer or glmer, Anova and pbkrtest functions in R). Multiple comparisons were performed using Tukey's honest significant differences method (lsmeans function).

Results

Costs of migration on survival and fitness-related traits

We collected 133 living and 67 dead individuals out of the 200 individuals initially introduced in the mesocosms. Mortality rate was higher in the lake (64%) than in the river (3%). Mortality rate was explained by the interaction between habitat of exposure and migration treatment (i.e. resident vs. migrant, $\chi^2_{df=1}$=4.522, p=0.03, Table 2, Fig.1). Specifically, the mortality rate of river migrants in lake was significantly higher than the mortality rate of lake residents, whereas migration treatment did not significantly explain mortality rates in the river.

Table 2: Generalized mixed linear model of mortality for lake and river stickleback ecotype transferred in lake or river habitat (df : degree of freedom, residuals df=192). Habitat refers to the habitat of exposure (lake or river) and migration to the experimental treatment (resident vs. migrant). Significant terms are highlighted in bold.

Effect	Df	χ2	P
Habitat	**1**	**23.78**	**<0.001**
Migration	**1**	**9.353**	**0.002**
Sex	1	1.449	0.229
Initial mass	1	1.739	0.187
Habitat: Migration	**1**	**4.522**	**0.034**

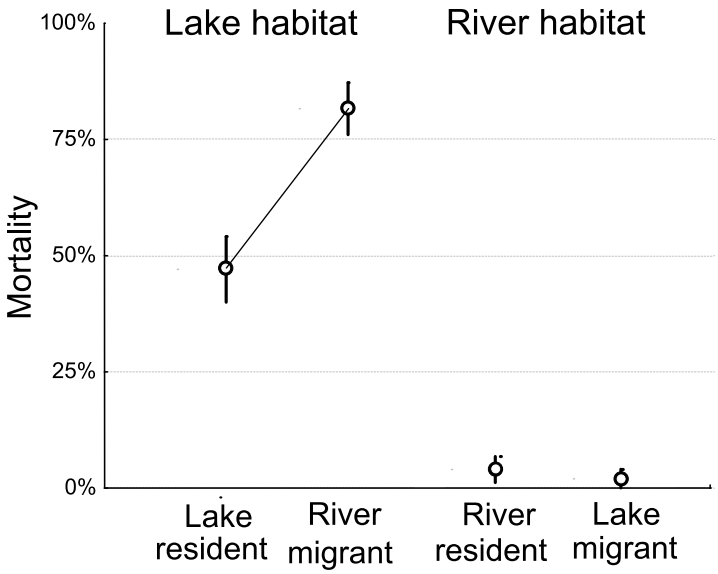


Figure 1: Mortality (expressed as the average proportion of dead fish +/- CI 95%) in lake and river stickleback ecotype in lake and river habitats. The mortality rate of river migrants was significantly higher than the mortality rate of lake residents ($\chi 2$=12.97, p=0.0001). The solid line represents a significant difference between migrants and residents in the lake.

Fish growth during the experiment was associated with fish sex and showed an interaction between habitat of exposure and migration treatment (Table 3): while in the lake, river migrants grew faster than lake residents, in the river, lake migrants grew slower than river residents (interaction: $F_{1,123.47}$=29.71, p<0.001, post-hoc tests: All p<0.0001, Table S1, Fig 2a). Overall, females grew faster than males ($F_{1,122.2}$=57.94, p<0.001).

Body condition was influenced by fish sex, habitat of exposure and migration treatment. Fish exposed to the lake habitat had a lower body condition than fish exposed to the river habitat (interaction: $F_{1,18.49}$=19.49, p=0.0003, Table 3, Fig. 2b). Overall, residents had higher body condition than migrants ($F_{1,123.92}$=4.9, p=0.029, Table 2, Fig. 2b).

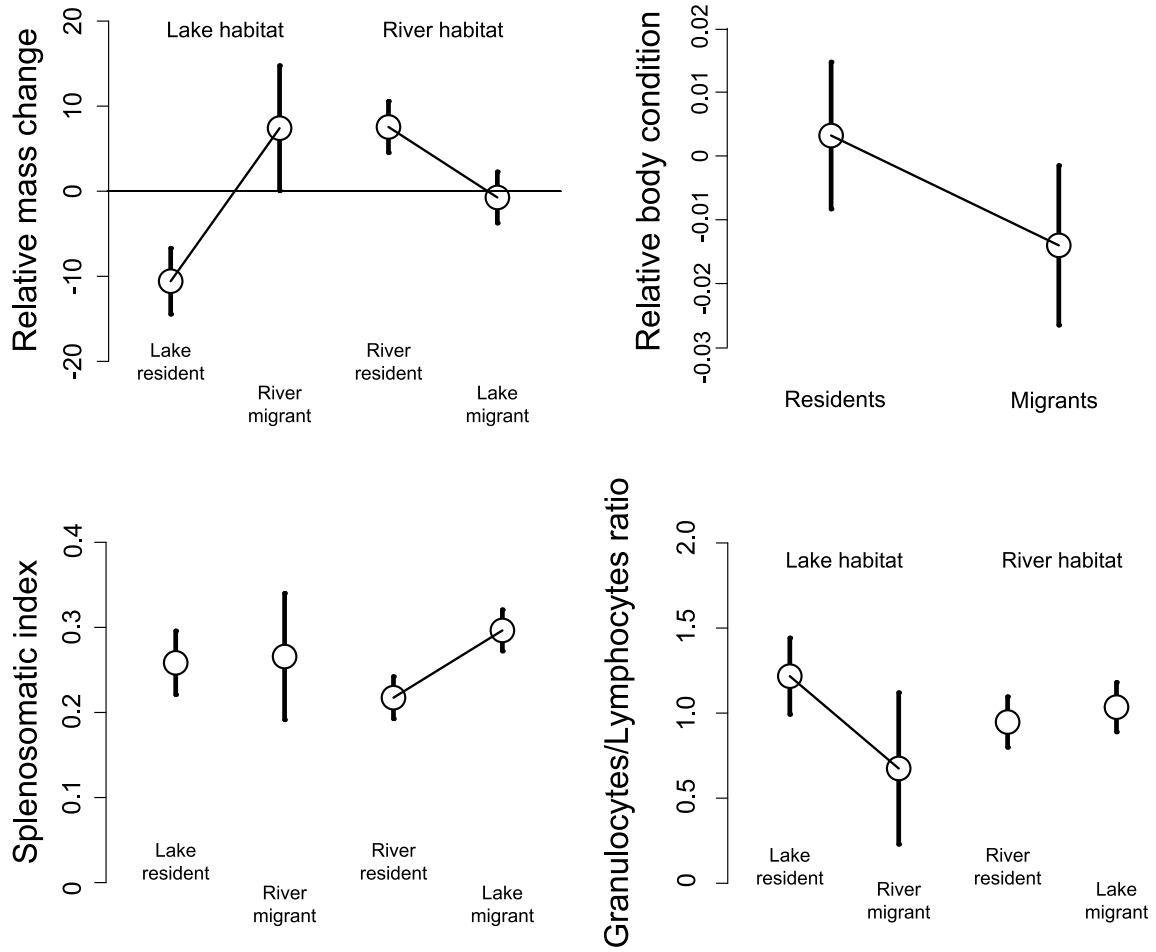


Figure 2: Differences in individual traits in lake and river sticklebacks transferred in lake and river habitats. Lines connecting means within habitat represent significant differences between residents and migrants (Tukey's HSD, $p<0.05$). Shown are least square means of the full models +/-0.95 CI for (a) relative mass change, (b) relative body condition, (c) Splenosomatic index and (d) Granulocyte to lymphocyte ratio. Solid lines represent significant differences between migrants and residents.

	(a) Relative mass change		(b) Body condition		(c) Splenosomatic index		(d) GL ratio	
	F	p-value	F	p-value	F	p-value	F	p-value
Habitat	**36.579**	**<0.001**	**19.491**	**<0.001**	1.491	0.237	0.431	0.517
Migration	**13.757**	**0.0003**	**4.903**	**0.029**	**26.869**	**<0.001**	0.355	0.553
Parasite load	0.317	0.574	0.443	0.507	0.225	0.636	**6.459**	**0.013**
Standard length (final)							3.889	0.051
Sex	**57.939**	**<0.001**	**25.703**	**<0.001**	**4.424**	**0.038**	0.299	0.585
Habitat:Migration	**29.713**	**<0.001**	2.639	0.107	**4.063**	**0.046**	**6.256**	**0.014**
Habitat: Parasite load	1.558	0.214	0.965	0.328	0.022	0.883	0.154	0.695
Migration: Parasite load	1.968	0.163	0.957	0.329	0.068	0.796	0.099	0.754
Habitat:Migration: Parasite load	**7.708**	**0.006**	0.356	0.552	0.011	0.916	1.76	0.187

Table 3: Analyses of variance tables of mixed effect models on condition related traits: (a) relative mass change (growth), (b) relative body condition and immune related traits: (c) splenosomatic index and (d) granulocyte to lymphocyte ratio. F-statistic were corrected with the Kenward-Roger approximation for mixed linear models.

Differences in parasite community between migrants and residents

We identified 19 different parasite taxa. The average number of parasite species per fish was 5.93 (range: 1-11, Table S2 and Fig. S2). Consistent with previous studies, individual parasite diversity and burden differed significantly between fish exposed to the two different habitat types, with higher diversity and burden in fish exposed to lake conditions compared to fish exposed to river conditions (diversity $F_{1,13.5}$=40.84, p<0.001; burden $F_{1,9.06}$=12.73, p<0.01, Table S3). Parasite diversity (i.e. Shannon index) was explained by the interaction between habitat of exposure and the migration treatment ($F_{1,110.6}$=5.198, p=0.025, Table S3, Fig.S3), indicating that differences in parasite diversity between residents and migrants were not the same between habitats of exposure. Parasite communities did not only differ between lake and river residents but also between lake and river migrants (multivariate permutational analysis, p=0.001; difference between lake and river residents R=0.656, p=0.0001; difference between lake and river migrants R=0.573, p=0.0001, Table S4, Fig. 3). In addition, experimental migrants differed from residents for both ecotypes (lake ecotype: R=0.357, p=0.0001, river ecotype: R=0.701, p=0.0001, Table S4, Fig. 4). The difference in parasites communities between migrants and residents was driven by three common parasites: the digeneans *Cyathocotyle prussica* and *Diplostomum sp.*, common in the lake, and the monogenean *Gyrodactylus sp.*, a common river parasite. These species respectively contributed 12.9 %, 10.8 % and 8.3%, respectively, to the dissimilarity between parasite communities in migrants and residents.

Contrasting immune responses between migrants and residents

Relative spleen mass was associated with the interaction between habitat of exposure and migration treatment ($F_{1,121.5}$=4.06, p=0.046 ; Table 3). Migrants in the river habitat had heavier spleens (post-hoc test: p<0.0001, Table S1, Fig. 2b), while no significant difference was found between migrants and residents in the lake habitat (post-hoc test: p=0.987, Table S1, Fig. 2b).

The granulocyte to lymphocyte (G/L) ratio was associated with the interaction between habitat of exposure and migration treatment ($F_{1,119.8}$=6.26, p=0.014, Table 2). Migrants in the lake habitat had a lower granulocyte to lymphocyte ratio than lake residents (post-hoc test: p=0.029, Table S1, Fig. 2c), while no significant difference was found between migrants and

residents in the river habitat (post-hoc test: p=0.931, Table S1, Fig. 2c). In addition, the relative proportion of granulocytes to lymphocytes, the G/L ratio decreased with parasite load over all experimental treatments ($F_{1,105.1}$=6.46, p=0.013, Fig S1).

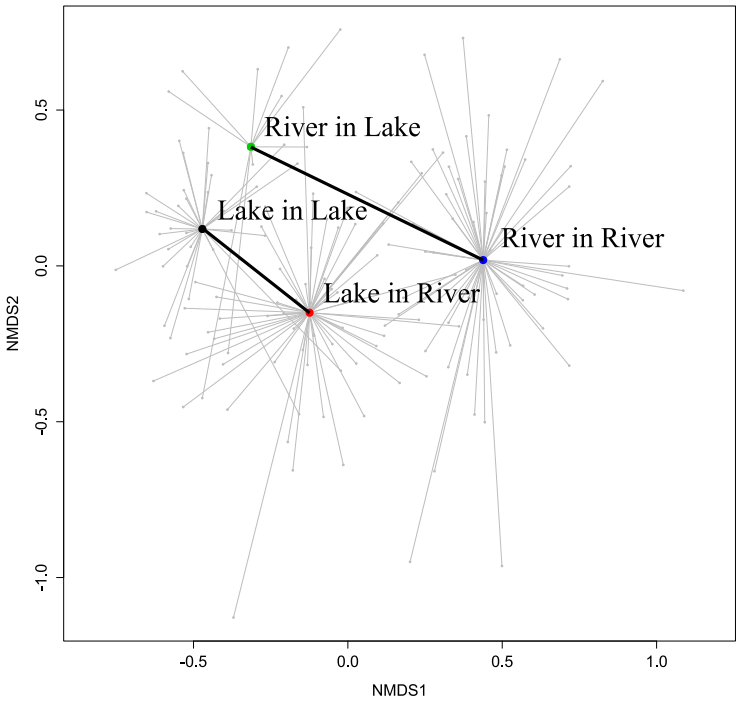


Figure 3:Non-metric multi-dimensional scaling plot based on community matrices of parasite abundances. Represented are lake and river three-spined sticklebacks transferred in their own habitat or in a different habitat. The distance between two points represents the difference in the overall parasite community between two individuals. (NDMS: non-metric multi-dimensional scaling axes). The length of the bold lines illustrates the extent of differences in macroparasite communities between lake and river residents and migrants.

The role of MHC genes

We detected 21 MHC haplotypes: 11 lake-specific, 7 river-specific and 3 shared by fish from both ecotypes (Table S10). The shared haplotypes displayed differences in prevalence between ecotypes (Fig. S4). The number of alleles per haplotype varied between one and three. Fish's MHC IIB differed significantly between ecotypes in terms of haplotype pools (ANOSIM, R=0.417, p=0.001), individual allele numbers ($\chi^2_{df=1}$=4.091, p=0.043) and allelic

divergence (t=2.55, p=0.012), with significantly higher allelic divergence and allele numbers in lake fish compared to river fish (see Fig. S4). An analysis of similarity identified the three haplotypes that contributed most to the difference between ecotypes in MHC IIB diversity: Haplotype G (16.1% contribution, So05.So11.SCX03, GenBank Accession Numbers DQ016402, DQ016404, and AJ230191), haplotype A (13.8% contribution, No13.No18, GenBank Accession Numbers AF395711 and AY687846) and haplotype F (11.9% contribution, No05, GenBank Accession Number AY687829). Even though both G and F haplotypes were shared between both fish ecotypes they differed in frequency between river and lake (G lake: 5.4%, river: 55.3%; F lake: 39.4%, river: 17.7%). The haplotype A was found only in the lake ecotype (prevalence: 48.4,%).

We found no significant differences in MHC diversity (allele number and allelic divergence) between experimental resident and migrant from each ecotype at the beginning of the experiment (all p>0.28, Table S6). Similarly, we did not find significant differences in MHC diversity or haplotype pools between dead and surviving residents and migrants in the lake (Permanova, p=0.708) or in the river (Permanova, p=0.273, Table S5 and S6).

Linking MHC IIB haplotypes and macroparasite infection

Overall, variation at the MHC IIB was tightly associated with the variation in parasites communities (Mantel r=0.162 p<0.001). MHC allelic divergence positively correlated to parasite diversity across treatments (Spearman's rho=0.214; p=0.019). However, this was likely due to differences in MHC and parasite diversity between treatments as we did not find significant associations between parasite diversity/burden and MHC diversity within habitats or treatments (all p>0.05, Table S3) We then tested associations between the three most divergent MHC IIB haplotypes and the three parasite species contributing most to differences between. We predicted associations between local haplotypes and the load of local parasites to change signs depending on the habitat of exposure. Infection load of the eye fluke *Diplostomum sp.* and the digenean *Cyathocotyle prussica* depended on the interaction between the presence of common lake haplotypes (A and F) and the habitat of exposure (*Diplostomum* : χ^2=35.376, p<0.0001 ; *Cyathocotyle* : χ^2= 59.903; p<0.0001 Fig. 4, Table S7). Fish carrying the haplotype A were both more resistant to these two parasite species in the lake and more susceptible in the river habitat (all post-hoc tests p<0.005, Table S8). Furthermore, fish carrying the haplotype F were found to have lower levels of infections with

Cyathocotyle prussica in the lake environment (χ^2=12.026, p=0.0005; Table S7 and S8, Fig. 4). These two parasite species were shown to be particularly virulent, as higher infection was significantly associated with reduced condition (e.g. Spearman correlation between growth rate and *Cyathocotyle prussica*: Rho=-0.27, p=0.003 or *Diplostomum sp:* Rho=-0.29, p=0.003; Table S9). Remarkably, we found that the infection load of *Diplostomum sp.* depended also on the interaction between the presence of the common river haplotype G and habitat of exposure, where the presence of this haplotype was associated with resistance in the river and susceptibility in the lake environment (χ^2=26.99, p<0.0001; Table S7 and S8, Fig.4). This suggests different host-parasite dynamics going on in the different habitat types. Interestingly, this river haplotype was also associated with susceptibility with the river monogenean *Gyrodactylus sp.,* whereas the lake haplotype A was associated with resistance to this parasite (Fig.4, Table S8).

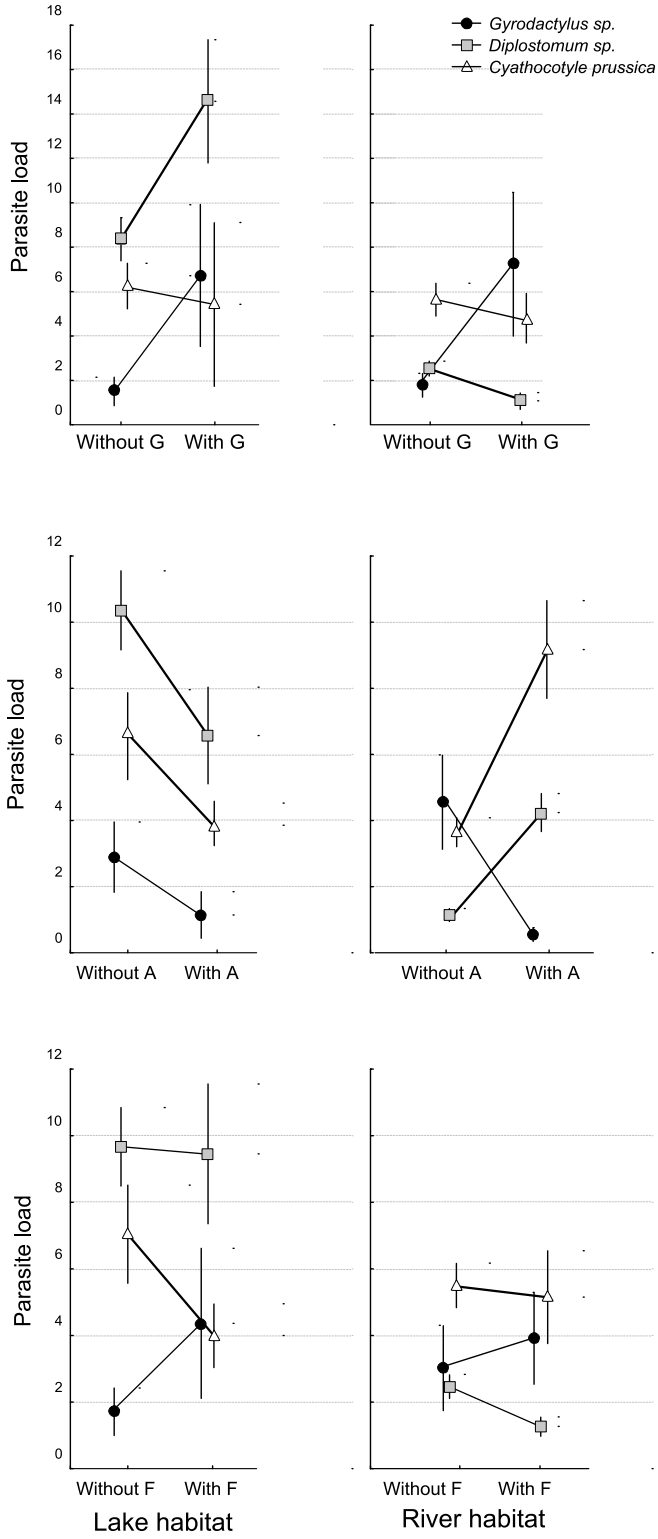


Figure 4: Parasite load of three parasites (circles: *Gyrodactylus sp.*, squares: *Diplostomum sp.*, triangles: *Cyathocotyle prussica*) in the lake or river habitat for individuals carrying common divergent MHCIIB haplotypes (a) haplotype G, (b) haplotype A, (c) haplotype F. Shown are means of linear models (± standard errors). Solid lines highlight combinations with significant interaction terms (Post-hoc odds ratio, $p<0.05$)

Discussion

The general goal of this study was to test for costs of migration as a potential reproductive barrier reinforcing incipient ecological speciation. Using a reciprocal transplant field experiment with wild-caught juvenile sticklebacks, we revealed strong selection against migrants in both river and lake habitats. We found strong asymmetric divergent selection between habitat types: while river migrants survived less in lake conditions, lake migrant grew much less than river residents under river conditions. Not only do we present differences in the functional innate immune responses between residents and migrants but also in the genetic basis of the adaptive immunity with direct evidence for habitat-specific patterns of resistance between common parasites and selected MHC haplotypes.

Costs of migration on survival and fitness-related traits

We show strong asymmetric effects of migration on fitness-related traits: While high immigrant mortality was found in the lake habitat (over 75% mortality rates for river migrants), in the river, both experimental migrants and residents experienced high survival rates. Using estimates of individual vigor, however, we also show a general cost for migrants on body condition under river conditions. Lake migrants had, compared to local river residents, both reduced growth and body condition. These results are consistent with a "local vs. foreign" pattern of local adaptation (Kawecki & Ebert 2004), where local fish would have a competitive advantage over migrants in their habitats. Particularly, relative lower body condition and smaller size would lead to reduced selectivity and attractiveness in mate choice for both male and female migrants (Wootton 1976; Milinski & Bakker 1990). It is then obvious that selection on those predictors of Darwinian reproductive success will prevent gene flow (Chellappa *et al.* 1995; Kalbe *et al.* 2009; Eizaguirre *et al.* 2012b). It is interesting to note that the effect on growth was only observed in the river habitat, while in the lake, migrants experimentally translocated from the river performed better than local fish residents. This discrepancy can be partially explained by the inherent differences between ecotypes, where river fish generally grow faster. Another possibility is that the selection that occurred favored the fastest growing individuals under lake conditions.

Our results are partly consistent with recent findings showing that migrant mortality was either asymmetric or absent in a lake–stream stickleback system in Canada (Räsänen & Hendry 2014). This suggests that even though parallel divergence is observed in multiple

lake-stream systems, the underlying pressures seem to be very local (Chain *et al.* 2014; Feulner *et al.* 2015). Although we cannot rule out that differences in growth between lake and river fish originates from multiple causes, the negative correlation found with parasite load suggests a cost of and therefore a role for parasite-mediated selection.

Habitat-specific parasite communities lead to asymmetric infection levels between reciprocal migrants

Local adaptation to parasites can contribute to the formation and maintenance of reproductive barriers during incipient ecological speciation (Summers *et al.* 2003; Eizaguirre *et al.* 2009a; Karvonen & Seehausen 2012), however the conditions under which it exists still remain elusive. By performing a reciprocal transplant experiment exposing wild-caught juvenile fish to different habitat types, we followed a realistic scenario where individuals dispersing most likely carry a habitat-specific parasite burden before dispersal. The two types of migrants (lake fish in river and river fish in lake) were harboring a combination of lake and river parasites, while being infected by different parasite communities. This potentially reflects seasonal differences in parasite abundance where migrants were exposed to different parasites early and late during their lifespan (Kalbe et al. 2002). In line with previous studies, exposure to the lake habitat increased the diversity of macroparasite infections, (e.g. Kalbe *et al.* 2002; Eizaguirre *et al.* 2011). Consequently, early infections in the lake and infections with foreign parasites in the river did not provide a complete release from parasite pressure for lake migrants. River fish migrating in the lake habitat experienced high levels of infections when compared to river residents, potentially leading to the observed costs. These high costs suffered by locally maladapted migrants facing foreign parasite communities could promote the evolution of reproductive barriers limiting dispersal to a different habitat and further reduce gene flow between habitats. This also reinforces the theory of local adaptation, as contrasting selective pressures in different habitats will improve fitness for residents relative to migrants. As migrants experience different selective pressures depending on their habitat of origin and of their new habitat, asymmetric gene flow could be reinforced between these habitats. There, the chance of finding healthy migrants is different in the lake and in the river habitat and might asymmetrically affect population structure between habitats (e.g. source-sink populations). Asymmetric gene flow, and resulting asymmetric introgression, between habitats would eventually lead to variation in the strength of selection for post-migration reproductive barriers between ecotypes and in the evolution of these barriers (Ryan & Wagner 1987; Hendry 2004; Nosil *et al.* 2005; Rafferty & Boughman 2006; Sobel & Chen 2014).

Experimental migration affects immune responses

Contrasting parasite communities exert divergent selection on the immune system, contributing to habitat-specific local adaptation and potentially to parasite-mediated speciation processes (Kalbe & Kurtz 2006; Eizaguirre *et al.* 2009a, 2011, 2012b; Karvonen & Seehausen 2012; Lenz *et al.* 2013; Scharsack & Kalbe 2014). In our system, local host maladaptation to parasites led to a complex interplay of innate and adaptive immune responses in migrants. We reveal that lake migrants showed an increased activation of the immune system, associated with higher levels of parasite infections as suggested by the negative relationship between the granulocyte to lymphocyte ratio and individual parasite load. We also found a lower proportion of granulocytes over lymphocytes in surviving river migrants. Again this might be due to selection for river fish with a relatively low level of innate immune response, as we showed that the cost of mounting an immune response induces associated fitness-costs (see also Bonneaud *et al.* 2003; Graham *et al.* 2011).

Maintenance of between-ecotype polymorphism at the MHC

Genes of the MHC provide the potential for local host adaptation to contrasting parasite communities (Eizaguirre *et al.* 2009a, 2012b; Eizaguirre & Lenz 2010). As a result of host-parasite coevolution, locally adapted MHC allele pools can lead to an optimal immune genetic makeup within each ecotype (Lenz *et al.* 2009b; Eizaguirre *et al.* 2011, 2012b). Habitat heterogeneity, together with host-parasite co-evolution, can favor specific resistance alleles in one habitat that ought to be linked to neutral or higher susceptibility in another with a contrasting parasite fauna (Hedrick 2002; Eizaguirre *et al.* 2011). We first confirmed with our study that lake fish harbor a higher mean number of MHC alleles than river fish. We also show that lake and river sticklebacks carry distinct MHC haplotype pools which correlate with parasite community across all treatments. More importantly, using translocation of individuals carrying habitat-specific haplotypes in contrasting environments, we show habitat-specific MHC haplotypes associated with resistance to local parasite, whereas the presence of the same haplotype is associated with susceptibility in the other habitat. We show this pattern for three common MHC haplotypes and two common virulent parasite species. Local adaptation to parasite communities via the rapid evolution of immunogenes (Eizaguirre *et al.* 2012a) can contribute to selection against migrants and can also lead to reproductive isolation.

Rapid selection of MHC allele pools is consistent with mechanisms of balancing selection maintaining MHC polymorphism within-population: Coevolutionary dynamics (such as Red Queen dynamics) can promote the maintenance of multiple variants (alleles, haplotypes) at variable frequencies within a given population and thus allows for the recycling of alleles by parasite-mediated selection (Woolhouse & Webster 2002; Bernatchez & Landry 2003; Milinski 2006; Eizaguirre & Lenz 2010; Eizaguirre *et al.* 2012a). Interestingly, some MHC haplotypes were associated with prevalence of several parasite species. Habitat-specific resistance to different generalist parasite species indicates that the exact same MHC haplotype can bind different parasite-derived antigens. This is likely to reduce selection for higher copy number of MHC loci, thus 1) avoiding costs of negative T-cell selection (Nowak *et al.* 1992; Woelfing *et al.* 2009) but 2) maintaining high levels of sequence divergence between alleles (Wakeland *et al.* 1990). Also, spatial variation in pathogen communities between habitats also contribute to the maintenance of MHC diversity at the species level (Hill 1991). Interestingly, previous studies have found different haplotypes associated with resistance to *Gyrodactylus spp.*, supporting the ideas of Red Queen dynamics acting on ecological time scale (Decaestecker *et al.* 2007; Eizaguirre *et al.* 2009b, 2011). In summary, we show that both within-habitat balancing selection and between-habitat fluctuating selection help maintain MHC diversity.

Costs against migrants has the potential to evolve when populations adapt locally to contrasting environments, and can thus represent a strong inherent barrier to gene flow before other pre-copulatory reproductive barriers arise in the context of ecological speciation. Barriers against migrants involved different traits (condition, survival) with different strengths of selection and this is likely to affect the evolution of further reproductive barriers. We show that parasites, via the activation of the innate immune system and contrasting patterns of resistance between habitats, are playing a key role in reproductive isolation in the early stages of ecological speciation.

Acknowledgements

We thank G. Augustin and D. Martens for their help maintaining the fish and for their help in the field. We thank S. Bartel, K. Brzezek, R. Derner, S. Liedtke, G. Schmiedeskamp and I. Schultz for invaluable help with dissections and laboratory assistance. We also thank the county of Schleswig-Holstein and the city of Ploen for permitting us to run the transplant experiments and the family Mahlzahn for allowing entrance to their property. All animal experiments were approved by the Ministry of Agriculture, Environment and Rural Areas of the State of Schleswig-Holstein, Germany.

Chapter III

Experimental parasite infection reveals costs and benefits of paternal effects

Published as:

Kaufmann, J., Lenz, T.L., Milinski, M. & Eizaguirre, C. (2014). Experimental parasite infection reveals costs and benefits of paternal effects. *Ecol. Lett.*, 17(11), 1409–1417.

Keywords: Host‐parasite interaction, in vitro fertilisation, paternal effects, sperm phenotype, three-spined stickleback.

Abstract

Forces shaping an individual’s phenotype are complex and include trans-generational effects. Despite low investment into reproduction, a father’s environment and phenotype can shape its offspring's phenotype. Whether and when such paternal effects are adaptive, however, remains elusive. Using three-spined sticklebacks in controlled infection experiments, we show that sperm deficiencies in exposed males compared to their unexposed brothers functionally translated into reduced reproductive success in sperm competition trials. In non-competitive fertilizations, offspring of exposed males suffered significant costs of reduced hatching success and survival but they reached a higher body condition than their counterparts from unexposed fathers after experimental infection. Interestingly, those benefits of paternal infection did not result from increased resistance but from increased tolerance to the parasite. Altogether, these results demonstrate that parasite resistance and tolerance are shaped by processes involving both genetic and non-genetic inheritance and suggest a context-dependent adaptive value of paternal effects.

Introduction

Understanding non-Mendelian modes of inheritance, such as parental effects, has become an important theme in evolutionary biology (Bonduriansky 2012; Rando 2012). Parental effects are defined as the influence of parental phenotypes on their offspring's phenotype beyond the direct effects of genetic inheritance (Mousseau *et al.* 2009; Wolf & Wade 2009). While increasingly acknowledged as an important factor, there is still controversy over the general adaptive value of parental effects (Marshall & Uller 2007). To be selected for, parental effects have to be on average at least slightly beneficial, however, on a short time scale, they can be beneficial to the parents, the offspring, both or neither of them (Marshall & Uller 2007). The adaptive value of a parental effect is expected to depend on the distribution of costs and benefits across parental and offspring generations and more importantly depends on the ecological context (Mousseau & Fox 1998; Marshall 2008). Adaptive parental effects are expected to evolve when the selective pressures are both variable and predictable (Burgess & Marshall 2014). Despite significant progress, the context-dependence nature of adaptive parental effects is still poorly understood. Furthermore, even though studies have mainly focused on maternal effects, there is growing evidence for variation in offspring phenotypes that may be attributed specifically to paternal effects (Mousseau & Fox 1998; Curley *et al.* 2011; Rando 2012). Studying paternal effects practically facilitates the experimental testing of adaptive non-genetic transmission, because, in contrast to the mother (e.g. through placenta, egg yolk, milk), the physiological links between father and offspring are generally very limited and can be more easily controlled (Curley *et al.* 2011; Rando 2012).

To assess the adaptive value and context-dependence of a paternal effect experimentally, it is necessary to manipulate exactly the same selective pressure in both parental and offspring generations. To this end, experimental exposure to parasites is ideal, given i) their ubiquitous presence in nature (Moore 2002) ii) their known fluctuating dynamics (Decaestecker *et al.* 2007), and iii) their detrimental effects on host condition and reproductive success (Schulenburg *et al.* 2009; Kalbe *et al.* 2009). Genes responding to parasite mediated-selection increase immunological resistance against the parasite and reduce the likelihood of infection (Sorci *et al.* 1997; Eizaguirre & Lenz 2010; Eizaguirre *et al.* 2012a). On the other hand, selection can also lead to increased tolerance of infection (Råberg *et al.* 2007; Sorci 2013). While some recent studies have shown that trans-generational immune priming can affect

survival, growth and immune responses during parasite or immune challenge (Gallizzi *et al.* 2008; Linder & Promislow 2009; Sadd & Schmid-hempel 2009; Roth *et al.* 2010, 2012), our understanding of the context-dependence of adaptive paternal effects and their consequences on resistance, tolerance and more broadly on host-parasite interactions are still poorly understood.

The three-spined stickleback (*Gasterosteus aculeatus* L.) is an established model species for studying the genetic basis of parasite resistance (Wegner *et al.* 2003a; Barber 2013) and its Mendelian inheritance (e.g. Eizaguirre *et al.* 2012). Here, we used this model species to investigate whether paternal effects can be expressed under experimental parasite pressure. Specifically, we estimated the effect of parasite exposure across two generations of three-spined sticklebacks, exposed to a standardized dose of a common stickleback parasite, the nematode *Camallanus lacustris*. We produced maternal half-sibships, each sired by one exposed and one unexposed male. The two sires of each half-sibship pair were brothers in order to reduce the well documented variation due to classical genetic inheritance. We then studied how paternal infection affected early life history traits and parasite resistance in the offspring generation. As males mainly contribute semen to the next generation, sperm represents the best candidate for functionally mediating paternal effects (Crean *et al.* 2012; Rando 2012, Bromfield et al. 2014). For this, we estimated variability of sperm traits under parasite infection and their consequences in competitive and non-competitive *in vitro* fertilization experiments.

Materials and Methods

Parasite exposure of lab-bred fathers

We dissected larvae of the nematode *Camallanus lacustris* from gravid female parasites collected from intestines of adult perches *Perca fluviatilis* from the vicinity of the stickleback population (Dieksee, 54° 9' 32.82", 10° 29' 47.63", Germany). This parasite is highly prevalent in the stickleback fish population (Kalbe *et al.* 2002; Eizaguirre *et al.* 2011), negatively affects their growth (Eizaguirre *et al.* 2012a), and is known to activate their immune system (Krobbach *et al.* 2007) as well as to select for resistance alleles at major histocompatibility complex genes (Eizaguirre *et al.* 2012a). As this parasite is trophically transmitted, we used copepods (*Macrocyclops albidus*) from a parasite-free laboratory culture

as intermediate hosts (van der Ven *et al.* 2000). We exposed groups of 100 copepods to 400 and 500 *C. lacustris* larvae for the paternal exposure and the offspring exposure, respectively. The number of larvae in the body cavity of each copepod was counted under a microscope to standardize the number of parasites each fish was exposed to. This manipulation guaranteed that the observed infection was directly linked to the immunocompetence of the fish and not confounded by the number of parasites the fish were exposed to (Eizaguirre *et al.* 2012a).

We bred ten full-sib families of three-spined sticklebacks, subsequently referred to as the G1 generation, by randomly pairing males and females from a natural lake population (Grosser Plöner See, 54° 9'21.16" N, 10°25'50.14" E, Germany). The fish from those families were kept under controlled laboratory conditions and were parasite-free at the beginning of the experiment. Male juveniles of each G1 family were randomly assigned to one of two treatments: parasite exposure or control (i.e. no exposure). We exposed males from the "exposure" treatment group twice to exactly six *C. lacustris* larvae (in copepods) whereas control males only received uninfected copepods. All G1 fish were transferred through artificial fall, winter and spring conditions in the lab in order to induce sexual maturation. Sixteen weeks after exposure, the G1 males (exposed and unexposed) were separated in single 16L aquaria with nesting material, while the G1 females were maintained in group aquaria (Sommerfeld *et al.* 2008). All individuals were fed *ad libitum* with frozen and live chironomid larvae. Males were inspected daily and nest quality of all males was evaluated following Jäger et al (2007). Only pairs (i.e. brothers) of reproductively active males (courting behaviour, each maintaining a nest of high quality for at least 2 days) were used in the experiments. For each trial, the selected males were sacrificed by a cut in the brain. After sperm collection (see below), the entire intestinal tract of each male was screened for *C. lacustris* under a dissection microscope (Kalbe *et al.* 2002). All exposed males were infected with at least one worm.

The parasite exposure treatment in the G1 generation could potentially result in an unintended and confounding selection bias in male quality between the treatment groups. This is because parasite exposure is known to affect mortality and reproductive behaviour. In order to control for this unintended bias, we tested whether more exposed than unexposed G1 males were excluded during the course of the experiment, for instance due to low nest quality. However, we did not find significant differences between exposed and unexposed G1 males in mortality, nest building behaviour, or the manifestation of courtship behaviour (all $p>0.49$; see supplementary table S1 in Supporting Information).

Sperm isolation and measurements

For both types of *in vitro* fertilization experiments, testes of G1 males were freshly dissected, weighed and transferred to a 40 µm micro-cell strainer sieve with 300 µl HBSS solution (Hank's Balanced Salt Solution, Sigma-Aldrich, Munich, Germany). Sperm suspension was prepared by gently mashing each testes through a cell strainer using a plastic stamp and rinsing the sieve twice with 300 µl HBSS solution. Three microliters of the resulting suspension were transferred to a counting chamber (standard count 4 chamber slide, 20 µm depth, Leja Nieuw Vennep, Nederlands) under an Olympus CX41 microscope at 100x magnification. To quantify spermatozoa concentration and velocity, we used computer assisted sperm analysis using a Hamilton-Thorne CEROS camera set-up and the Animal Mobility software (Hamilton Thorne Biosciences, Beverly, MA, USA). We recorded the total number of sperm, motile sperm number and the following sperm motion parameters: Beat-cross frequency (BCF) as well as curvilinear (VCL), straight-line (VSL) and average-path velocity (VAP) (Kime *et al.* 2001). We recorded 6 measurements of each sperm characteristic per individual (3 separate areas from each of two slide chambers) and used the average value in subsequent analyses.

In vitro sperm competition experiments

To test for the consequences of parasite exposure on the functional variation of fertilization, we prepared 15 sperm competition assays between sperm extracted from one exposed and one unexposed male of the same lab-bred G1 family. Using brothers for these experiments reduces the effect of genetic variation on sperm phenotypes, sperm competition outcome and offspring phenotype. For each test, we fertilized the eggs of a random female (taken from the same lab-bred G1 generation but not from the males' family) with 50 µl of sperm solution from each of the two brothers in 5ml of fresh water. Using the same individuals, we also performed matched sperm competition assays where total sperm concentration was adjusted to the lowest concentration of the two males. Five days after fertilization, the eggs were sampled for DNA analysis. DNA extraction was performed using the Invisorb® DNA Tissue HTS 96 Kit (Invitek, Berlin, Germany) on a TECAN FreedomEvo robot platform. All eggs were genotyped at 15 microsatellite loci (Kalbe *et al.* 2009) for paternity analysis (n=1157, mean of eggs per test 39±14 S.D.). We performed genotyping using GeneMarker 1.85

(Softgenetics LLC, State College, PA, USA) and individual parentage analysis using CERVUS v3.0.3 (Field Genetics Ltd, Kalinowski 2007). The most likely father was determined based on exclusion probabilities and LOD score ratios between the two putative sires (mean paternal assignment of 93.38 %).

Production of offspring generation

In order to test for the impact of parasite exposure on fertilization success and for paternal effects *per se*, we also performed *in vitro* fertilizations in a non-competitive split-clutch design. Each maternal half-sibship pair was mothered by one G1 female and sired by two G1 brothers, one exposed and one unexposed. To control for potential family effects, the parents originated from a total of 10 different G1 laboratory-bred families. In total, we produced 53 maternal half-sibship pairs (range: 2 to 9; average: 5 per G1 family), subsequently referred to as the G2 generation. For this we randomly selected a G1 gravid female (not the same family as the males') and two reproductively active brothers. The females' eggs were stripped carefully into a dry sterile Petri dish (90x15mm). We divided each clutch evenly into two halves: One half was fertilized with 100 µl of sperm solution from the G1 male exposed to the parasite and the other half was fertilized with 100 µl of sperm solution from the unexposed male. Eggs and sperm were left for 20 minutes at 18°C to assure complete fertilization. Five days after fertilization, we counted the number of developing, unfertilized and undeveloped eggs under a laboratory microscope. We characterized unfertilized eggs by the sole presence of lipid droplets and undeveloped eggs by a delayed developmental stage as well as the absence of a heartbeat five days post-fertilization (supplementary figure S1).

Offspring care and exposure

To estimate juvenile mortality in the G2 generation, we monitored the presence of dead juveniles at least three times a week for 6 months. After this period, we randomly selected 15 maternal half-sib G2 families (representing 5 out of the 10 initially produced G1 families) to challenge them with the same nematode parasite as the G1 fathers. For this, we randomly assigned fish of both sexes from each G2 family either to the parasite exposure treatment (9-10 fish per family) or to the control treatment (5-6 fish per family). The total number of fish was 475. Prior to the experimental treatment, fish were measured, weighed and a spine was clipped for later identification. The methods of G2 parasite exposure and fish dissections were

strictly the same as in the parental G1 generation except that G2 offspring from the "exposure" treatment were exposed to exactly seven C. lacustris larvae each. We then transferred them in groups of 26 fish to 16L tanks, mixed by paternal treatment, experimental treatment and family to avoid confounding tank effects. We used DNA fingerprinting based on 11 microsatellite loci (Kalbe *et al.* 2009) on spine and fin samples (before and after treatment, respectively) to identify G2 individuals with their respective treatments at the end of this double-blind experimental set-up. All G1 and G2 fish were laboratory-bred and thus parasite-free before exposure to *C. lacustris*.

Statistical analysis

Effect of parasite exposure on sperm phenotype and functional competitiveness

All statistical tests were conducted in R v 3.0.3 (R Development Core Team 2014). We tested differences in testes mass and sperm characteristics (velocities and concentration) between exposed and unexposed males using a linear model with treatment and testes mass as fixed effects. We estimated the paternity of the unexposed G1 male in each of the 15 sperm competition trials and tested this value against 50 % (representing random fertilization) using one sample t-tests.

Cost of paternal exposure on offspring early life history traits

The proportion of unfertilized and undeveloped eggs was calculated over the total clutch size. Juvenile mortality was calculated based on the total number of dead G2 juveniles over the initial number of developed eggs per clutch. We tested differences in the proportion of unfertilized eggs, undeveloped eggs and juvenile mortality between clutches sired by exposed or unexposed G1 males using non-parametric Wilcoxon signed-rank tests (wilcox.test function in R).

Effect of paternal exposure on offspring resistance

Resistance is defined as the ability of hosts to supress the establishment of parasites and thus limit parasite load (Råberg *et al.* 2009; Sorci 2013). We tested the effects of paternal G1 exposure on the likelihood of infection (infected vs. uninfected, $n_{exposed}$=223) and on infection intensity (number of worms in infected individuals) in the G2 fish using generalized linear mixed effect models (glmer function in R). The full model included sex, G2 size before exposure and paternal G1 treatment (exposed vs. control) as fixed effects, and maternal G2

half-sibship identity as random effect to account for non-independence between the two paired maternal half-sibships. Infection probability was fitted with a binomial (log-odds link function) distribution and infection intensity fitted with a Poisson distribution (log link function). The significance of the paternal effect was tested by comparing models with or without the paternal G1 treatment variable using likelihood ratio tests (using anova function in R). We did not find evidence for over-dispersion in our models (supplementary table S2).

Effect of paternal exposure on offspring tolerance

Tolerance is defined as the ability of hosts to limit the physiological costs caused by a given parasite burden or, *sensu stricto*, as the reaction norm of host fitness and condition over parasite burden (Råberg *et al.* 2009; Sorci 2013). In our experiment, parasite-related paternal effects could not only be expressed through resistance but also through increased tolerance, where G2 fish sired by exposed G1 males would suffer less from parasite-induced fitness consequences than their counterparts sired by unexposed G1 males. We thus tested whether infection with *C. lacustris* affected body condition in G2 fish differently with respect to the paternal G1 treatment. Body condition of the G2 fish, an estimate of fish health and a predictor of energy reserves and reproductive success, was calculated using the residuals from the regression of body mass on body length (Chellappa *et al.* 1995). The linear mixed effect model (nlme function in R) included G2 body condition as dependent variable, sex, G2 treatment (exposed vs. control), paternal G1 treatment (exposed vs. control), and their interactions as fixed effects as well as maternal G2 half-sibship identity as a random effect.

Fish dissection showed that approximately half of the exposed G2 fish did not harbour any parasite at the end of the experiment. Since the actual moment of infection and the continuing interaction with an established parasite are two inherently different processes, we hypothesized that the cost of parasite infection might be very different between infected and exposed-but-uninfected G2 individuals. Hence, we tested the effect of paternal G1 exposure on tolerance only in exposed G2 offspring, split for exposed-uninfected and exposed-infected offspring. For this we ran the same linear mixed effect model on G2 body condition as above, but focusing only on exposed G2 fish (exposed-uninfected vs. exposed-infected) instead of all fish.

To investigate in which way paternal G1 exposure affected offspring tolerance, we tested how the relationship between G2 body condition and infection intensity was affected by paternal G1 exposure. This was tested in a linear mixed model on G2 body condition with paternal G1

treatment and the interaction between paternal G1 treatment and G2 infection intensity as fixed effects. Maternal half-sibship identity was set as a random effect.

Results

Effect of parasite exposure on sperm phenotype and functional competitiveness

All exposed G1 males were infected with at least one parasitic worm. We did not find significant differences in testes mass, total sperm concentration or measures of sperm velocity between exposed and unexposed males (testes mass: p=0.13; total sperm concentration: p=0.07; all velocities: p>0.2). However, motile sperm concentration was found to be significantly lower in exposed males than in unexposed males ($F_{1,122}$=4.595, p=0.034; Fig. 1). This difference translated into an advantage for the unexposed G1 males, which fertilized on average 65.65% of the eggs. This value was significantly higher than an evenly shared paternity ($t_{df=14}$=2.181, p=0.023), but not so when total sperm concentration was experimentally matched between brothers ($t_{df=13}$=1.003, p=0.167). These results suggest a reduction in the concentration of motile sperm in response to infection (Pearson's correlation between total and motile sperm concentration: r=0.908, p<0.001).

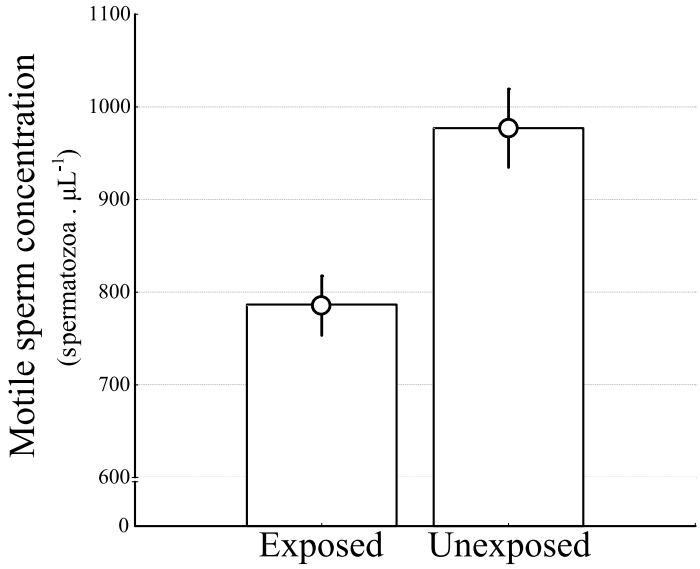


Figure 1: Parasite infection induces sperm deficiency. Concentration of motile spermatozoa per µL in male sticklebacks experimentally infected with the nematode *Camallanus lacustris* and in uninfected (unexposed) males. Error bars represent ±1 SE

Cost of paternal exposure on offspring early life history traits

In non-competitive fertilization trials we did not observe significant differences in fertilization rates between clutches sired by exposed or unexposed males (Wilcoxon signed-rank test: n=53, T=181, Z=0.192, p=0.848). However, eggs fertilized by G1 males that were exposed to parasites suffered higher rates of developmental failures than the ones fertilized by unexposed G1 males, resulting in lower hatching success (Wilcoxon signed-rank test: $n_{clutches}$=53, n_{eggs}=4316, T=157, Z=2.765, p=0.006; Fig.2a). Furthermore, larvae of exposed G1 males also showed a higher mortality rate (Wilcoxon signed-rank test: $n_{clutches}$=50, $n_{developed\ eggs}$=3602, T=158.5, Z=2.912, p=0.004; Fig.2b). Motile sperm concentration, zygote and juvenile mortality were not significantly correlated among each other (see supplementary table S3).

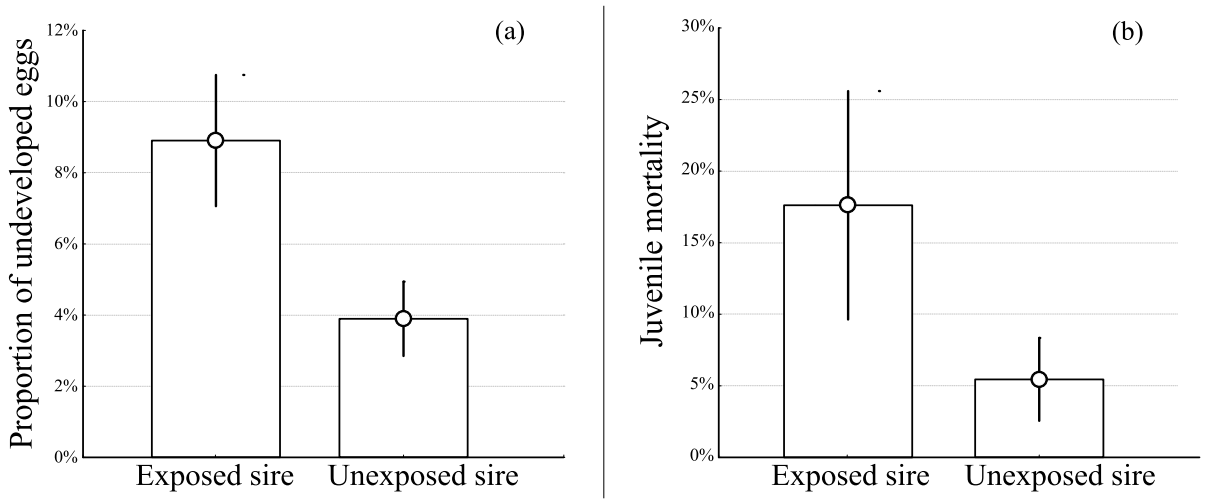


Figure 2: Transgenerational effects of paternal parasite infection on (a) the proportion of undeveloped eggs and (b) the proportion of dead juveniles in maternal half-sibships sired by exposed or unexposed fathers. Error bars represent ±1 SE.

Effect of paternal exposure on offspring resistance

We found no significant differences between surviving G2 individuals sired by exposed or unexposed G1-males in their probability to become infected when exposed to the same parasite as the paternal generation (likelihood ratio test [LRT], $n_{exposed}$=223, χ^2_1=3.599, p=0.165, supplementary table S4 and supplementary figure S2) or in infection intensity (the number of parasites when infected, LRT, $n_{infected}$=113, χ^2_1=0.061, p=0.97, supplementary table

S5). Notably, 43% of the variation in the likelihood of being infected was attributable to maternal half-sibship origin.

Effect of paternal exposure on offspring tolerance

Prior to experimental treatment, offspring sired by unexposed males had higher body condition than offspring sired by exposed males ($F_{1,358}$=4.32, p=0.038). After the experimental exposure period, we found significant effects of paternal G1 treatment and G2 treatment on G2 body condition. On the one hand, offspring sired by exposed G1 males achieved a higher body condition than their counterparts sired by unexposed G1 males ($F_{1,471}$=8.74, p=0.003; supplementary table S5 and supplementary figure S3). On the other hand, experimental parasite exposure significantly reduced body condition in G2 fish ($F_{1,471}$=6.42, p=0.012; supplementary table S6, Fig. 3). Noteworthy, 38% of the variation in body condition at the end of the experiment was attributable to maternal half-sibship identity.

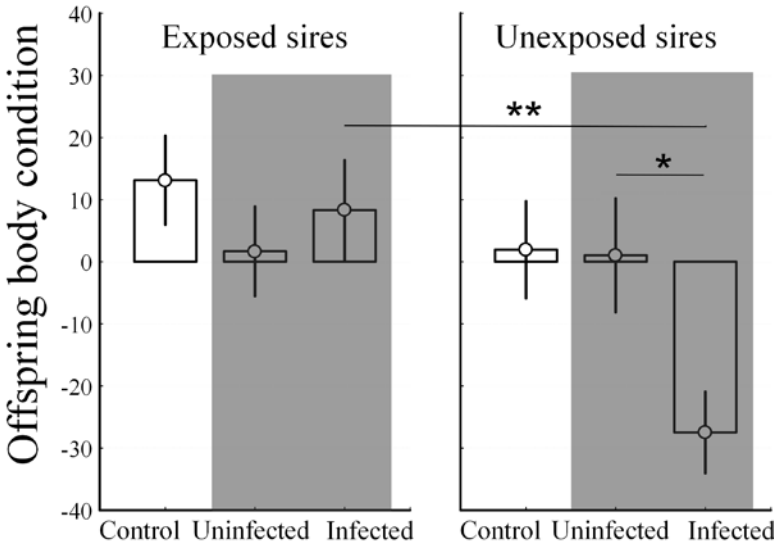


Figure 3: Trans-generational effects of paternal parasite exposure on body condition at the end of the experiment. Body condition is an estimate of fish health and is calculated using the residuals from the regression of body mass on body length. Shown are means of body condition in control, uninfected (i.e. exposed but non-infected) and infected offspring, sired by either exposed or unexposed fathers. Error bars represent ±1 SE. The shaded data indicates the comparison of exposed-uninfected and exposed-infected fish. Symbols represent significant differences between experimental groups based on Tukey post-hoc tests (* : p=0.048; ** : p=0.003).

As exposed G2 fish encompassed both infected and uninfected individuals in approximately equal proportions, we additionally focused on the variation in G2 body condition in response to G1 paternal effects between exposed-infected and exposed-uninfected individuals. Here we found a significant interaction between paternal G1 treatment and G2 infection status on G2 body condition ($F_{1,282}$=4.14, p=0.043; Table 1, grey shades in Fig. 3): G2 fish sired by unexposed males suffered significantly from the cost of parasite infection (Tukey post-hoc test, z=2.58, p=0.048), whereas G2 fish sired by exposed males did not (Tukey post-hoc test, z=-0.25, p=0.995). This result seemed to be mainly driven by infected G2 fish sired by unexposed G1 males, which showed a significantly lower body condition than their counterparts from exposed G1 males (Tukey post-hoc test, z=-3.47, p=0.003, Fig. 3), while paternal G1 exposure did not significantly affect body condition in uninfected individuals (Tukey post-hoc test, z=-0.71, p=0.894). This suggests that beneficial effects of paternal exposure are only expressed in offspring upon challenge by the selective parasite.

Table 1: Effects of paternal exposure, offspring infection status (infected vs. exposed but uninfected) and sex on individual body condition. The statistical table shows the outcome of a linear mixed model on individual body condition at the end of the experiment. The variation imputed to the random effect was estimated based on the ratio of the variance due to this effect over the total variance (d.f..: degrees of freedom)

Effect	d.f.	F value	P
Paternal exposure	**1, 282**	**8.161**	**0.005**
Offspring infection	1, 282	2.551	0.111
Offspring sex	1, 282	0.505	0.478
Paternal exp. x Offspring infection	**1, 282**	**4.144**	**0.043**
Maternal half sibship (random effect)	Variance = 36.44%		

To further dissect this effect, we investigated tolerance as the relationship between offspring body condition and infection intensity, with respect to paternal exposure. We found a significant interaction between paternal G1 exposure treatment and the number of established parasites in G2 fish on body condition ($F_{2,281}$=4.11, p=0.017, Table 2, Fig. 4): G2 fish sired by unexposed G1 males showed a decrease in body condition with increasing number of parasites (estimated slope=-8.39; 95% CI=-14.6 to -2.2; t=-2.84, p=0.005) whilst body condition of fish sired by exposed G1 males appeared relatively unaffected by parasite infection (estimated slope=0.15; 95% CI=-5.1 to 5.4; t=-0.53, p=0.59).

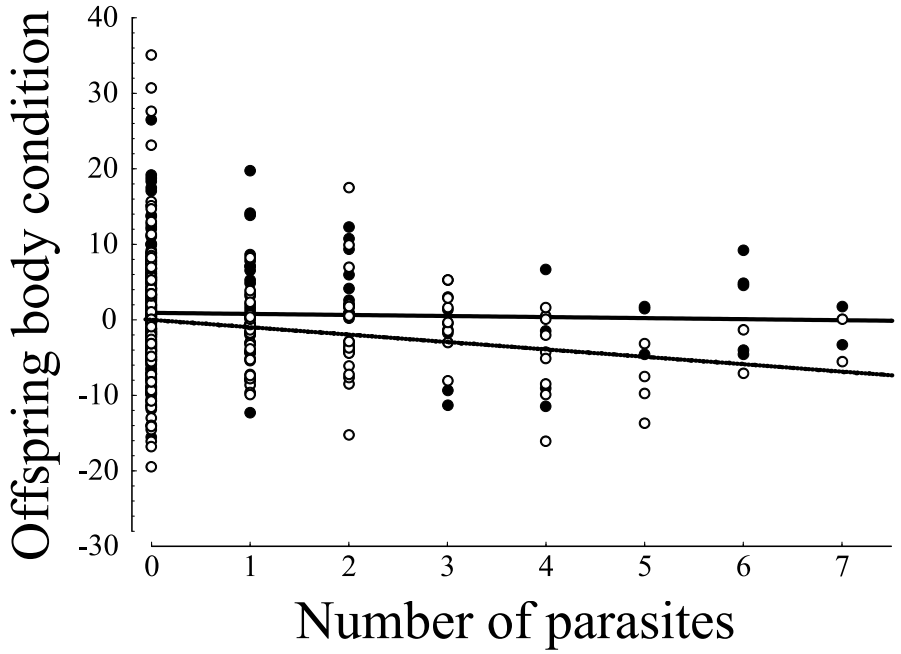


Figure 4: Trans-generational effects of paternal parasite exposure on the relation between offspring body condition and infection intensity (i.e. tolerance). The black circles and the solid linear regression line represent exposed fish sired by exposed fathers and the white circles and the dashed linear regression line represent exposed fish sired by unexposed fathers.

Table 2: Effects of paternal exposure and offspring infection intensity (number of established parasites) on individual body condition. The statistical table shows the outcome of a linear mixed model on individual body condition at the end of the experiment. The variation imputed to the random effect was estimated based on the ratio of the variance due to this effect over the total variance (d.f..: degrees of freedom)

Effect	d.f.	F value	P
Paternal exposure	**1, 281**	**9.292**	**0.003**
Paternal exp. x Offspring infection intensity	**2, 281**	**4.116**	**0.017**
Maternal half sibship (random effect)	Variance=36.88%		

Deciphering selection from increased parasite tolerance

The difference in tolerance of G2 fish with respect to paternal treatment indicates the existence of mechanisms induced by paternal infection. It could, however, also result from selection against low quality G2 individuals during early life. Such a selection scenario could have resulted in an elevated average quality of the surviving G2 fish sired by exposed G1 males, and could in turn explain their elevated tolerance to parasite infection. In order to control for this scenario, we repeated the same statistical model as presented in Table 1, but we simulated selection by excluding G2 offspring sired by unexposed fathers across a range of selection strengths varying from 5 to 34.8 % (the latter corresponding to twice the relative difference in overall survival between offspring from exposed and unexposed fathers, i.e. 17.4%). With these sensitive analyses, we simulated scenarios postulating (i) selection against weaker G2 offspring sired by infected father (supplementary table S7a) and (ii) random selection independently of infection (supplementary table S7b, S7c) to account for the effect that selection may not have exclusively removed the most susceptible individuals. In each case, based on 999 simulated subsets, we estimated the mean p-value and 95% confidence interval of the paternal effect and the interaction between paternal exposure and offspring infection on offspring body condition. Paternal G1 treatment on offspring body condition remained significant, even at high levels of simulated random and infection-dependent selection ($p<0.047$, supplementary table S7). The interaction (G1 exposure x G2 infection) was supported by a statistical trend up to 20% contribution of selection (maximal mean p-value=0.08, Supplementary table S7). Altogether, these analyses support the conclusion that

selection in offspring of exposed fathers at the juvenile stage was not the only source for differences in offspring condition in our experiment, as well as corroborate a potential context-dependence for the benefits of this paternal effect. Lastly, we show that infection cost (i.e. mean difference in body condition between infected and uninfected individuals per family) did not significantly correlate with offspring mortality (see supplementary table S3).

Discussion

In addition to traditional genetic inheritance, parental effects are potent processes than can alter offspring phenotypes (Marshall & Uller 2007; Bonduriansky 2012; Burgess & Marshall 2014). Here, we present compelling experimental evidence for trans-generational effects of paternal parasite exposure on juvenile survival and offspring condition. While offspring of exposed sires generally suffered from reduced juvenile survival, suggesting parasite-mediated selection, the surviving offspring showed a significantly higher body condition than their counterparts from unexposed fathers. Interestingly, our in depth analyses revealed that a fine-tuned interaction between selection and parental effects may result in a context-dependent advantage of this trans-generational effect where effects are strongest when both parental and offspring generations are exposed to similar selective pressures.

Firstly, not only had exposed males lower motile sperm concentration than unexposed males, but this also resulted in a lower rate of paternity in competitive situations. When both total sperm concentration and, as a result, the concentration of motile sperms were adjusted, differential fertilization success was not observed anymore, demonstrating that this trait is condition-dependent and represents a key functional link between infection and reproductive success during sperm competition. Secondly, in non-competitive *in vitro* experiments, male infection resulted in increased reproductive failures and lower probability for the offspring to reach adulthood. This result demonstrates that parasite exposure affects fertilization and post-fertilization development and although poor quality sperm can fertilize eggs in a non-competitive interaction, carry-over effects can then exist. Altogether, we demonstrate strong sperm-mediated trans-generational costs of parasite infection on reproductive success. These results are consistent with i) studies showing that the activation of the immune system upon stimulation decreases sperm velocity and fertilization success (Chargé *et al.* 2010; Losdat *et al.* 2011), ii) the *sick sperm* hypothesis, where paternal stress can alter sperm phenotype and

affect post-zygotic development and performance (Crean *et al.* 2012, 2013; Rando 2012; Zajitschek 2014; Bromfield *et al.* 2014). Furthermore, zygote and juvenile mortality did not correlate among families. Whether this suggests several independent mechanisms or an interaction between genetic background and the expression of trans-generational costs on reproductive success remains an open question. Even though the direct mechanisms of sperm mediated effects here are not clear, they may be associated with the release of reactive oxygen and nitrogen radicals which can damage proteins, lipids, DNA and can disrupt mitochondrial function (Sorci & Faivre 2009).

Using male siblings in our experimental design, we minimized the potential effects of classical genetics, e.g. through the inheritance of resistance alleles (Eizaguirre *et al.* 2012a) and demonstrate that a large proportion of the observed costs originated from non-genetic paternal effects. To control for the possibility that treatment-induced selective mortality may have acted specifically against weaker offspring sired by exposed fathers and thus biased their mean intrinsic quality independent of paternal effects, we simulated varying levels of selection. While selection prior to parasite exposure of the offspring has probably acted in our experiment, our analyses also support the observation that increased body condition is associated with infection-induced paternal effects. Moreover, increased tolerance was not associated to high levels of mortality at the family level. Thus, both parasite resistance and tolerance are likely shaped by processes involving both genetic and non-genetic trans-generational effects.

Whether and when paternal effects are adaptive remain open questions in the literature (e.g. (Uller *et al.* 2013). For paternal effects to be adaptive and thus get selected for, the benefits would have to outweigh the associated costs. In this study, we show that paternal infection can have significant costs ranging from deficient sperm to juvenile mortality, but paternal infection can also have clear beneficial effects on offspring condition, leading to a compensatory increase in Darwinian fitness of exposed fathers. The significant cost of infection in offspring sired by non-infected males is likely to lead to their competitive disadvantage against offspring sired by infected males, particularly as body condition is an accurate measurement of energy reserves and mate quality in sticklebacks (Milinski & Bakker 1990; Chellappa *et al.* 1995; Jakob *et al.* 1996). With our experimental design we could also test the hypothesis that adaptive paternal effects are context-dependent, i.e. expressed when both the paternal and offspring generations are predictably exposed to the same selective pressure. Burgess and Marshall (2014) recently stressed the importance of environmental

predictability in the study of adaptive paternal effects. At least in our stickleback populations, the presence of a parasite in a given generation is more likely to predict parasite presence in the next generation than to predict parasite absence in the next generation (Kalbe *et al.* 2002; unpublished data). The fact that paternal effects are only observed in actually infected offspring may be due to the favourable lab conditions under which fish were kept and where costs associated to solely mounting an immune response (without the continuous costs of parasite infection) may be compensated for (Jäger *et al.* 2007). Nonetheless, our study suggests that under predictable selective pressures that impact both parental and offspring generations (such as parasite infection), trans-generational effects can be adaptive.

Interestingly, the paternal effects were not expressed as increased resistance to the parasite, but rather as a difference in body condition, resulting from increased tolerance (Råberg *et al.* 2007; Sorci 2013). In our experiments, offspring body condition had both a genetic and non-genetic trans-generational component, while the probability of infection and the level of infection strongly depended on the family background (i.e. classical genetics). Although our experimental design significantly reduced genetic variation, we still show that this variation played a major role in the individuals' response to parasite infection.

There is substantial evidence for mechanisms of non-genetic inheritance, such as the inheritance of epigenetic alterations or the transmission of proteins or molecules from the parent to the offspring (Bonduriansky & Day 2009; Kappeler & Meaney 2010; Jiang *et al.* 2013). As males are more limited in their possibility to transmit information and resources than females (Curley *et al.*, 2011), we expect trans-generational paternal effects to be mainly mediated through epigenetic changes in the germ line. Ultimately, epigenetic changes can also affect selection, e.g. by allowing for a more plastic and more immediate response to selection than classical genetic mechanisms of inheritance (Klironomos *et al.* 2013a). Furthermore, parental effects may buffer selection at the genetic level. This can allow for the short-term maintenance of otherwise neutral or even slightly deleterious alleles, potentially promoting allelic diversity at genes involved in the response to fluctuating selective pressures, alongside traditional processes of long-term balancing selection. This can influence many processes such as population extinction, speciation and specifically host-parasite co-evolutionary dynamics (Bernardo 1996; Wolf *et al.* 1998).

Acknowledgements

The authors thank G. Augustin and D. Martens for their help maintaining the fish, M. Schwartz and R. Leipnitz for their help in the copepod work and N. Wildenhayn, W. Derner, G. Schmiedeskamp and Henrike Schmidt for their help in lab procedures. We would like to thank M. Kalbe for helpful advices as well as the fishermen from Niederkleveez for their assistance in the acquisition of fresh perch guts. The authors thank D. Marshall and three anonymous referees for constructive comments that improved the manuscript. All animal experiments were approved by the Ministry of Agriculture, Environment and Rural Areas of the State of Schleswig-Holstein, Germany.

Conclusion

In this thesis I outlined the role of parasites in adaptive evolution and particularly explained how parasite-mediated selection contributes to the evolution of host defenses and reproductive barriers. My PhD projects focused on (i) exploring the role of gametic isolation in ecological speciation, (ii) testing the role of local adaptation to parasites in ecological speciation and (iii) unravelling the role of paternal non-genetic effects of infection.

I will now briefly present the outcomes of my PhD work and how they improve our understanding of parasite-mediated selection and parasite-mediated speciation. I will also suggest how these findings could be complemented further.

The first part of my Ph.D. work addressed the importance of specific reproductive barriers during the early stages of ecological speciation. Previous work already suggested the role of parasites limiting gene flow between lake and river populations in Northern Germany (Kalbe *et al.* 2002). Specifically, differences in macroparasite communities between these ecological demes, or ecotypes, lead to habitat-specific MHC class II allele pools (Eizaguirre *et al.* 2011). MHC-mediated local adaptation to parasites, reinforced by MHC-mediated assortative mating, keeps gene flow reduced between these populations (Eizaguirre *et al.* 2009a, 2011, 2012b). To broaden our understanding of the system, I tested for the existence of postcopulatory barriers in the form of conspecific gamete preference and gametic incompatibilities and for parasite-mediated costs of migration as a precopulatory barrier.

In chapter I, I examined post-copulatory reproductive barriers and specifically sperm phenotypes and assortative gamete precedence between ecotypes. There I demonstrated that sperm phenotypes consistently differed between lakes and rivers but that these differences did not lead to ecotype-specific sperm precedence in sperm competition experiments. However, the link between zygote mortality and paternity under these conditions suggests that genetic incompatibilities might have arisen between lake and river populations and contribute to reinforce the onset of speciation.

Interestingly, the pleiotropic role of MHC on parasite resistance and mate choice can also be applied to gametic processes. Indeed, MHC-mediated cryptic female choice is theoretically

possible, although support for this idea is extremely scarce and so far inconclusive (Wedekind *et al.* 1996; Yeates *et al.* 2009; Alcaide *et al.* 2012; Løvlie *et al.* 2013). Therefore, local adaptation to different habitats characterized by different parasites might also be accelerated by cryptic female choice for MHC alleles conferring resistance to local parasite fauna. Future studies on gametic processes involved in speciation could therefore focus on MHC-mediated cryptic female choice.

In chapter II, I showed that migrants between habitats suffer from lower survival rates or reduced condition. The strong effects of experimental migration on 1) the parasite communities harbored by lake and river fish and 2) the balance between adaptive and innate immune systems suggest that parasites are a major ecological factor for maintaining reproductive isolation between lakes and rivers. This was further supported by the fact that I found habitat-specific patterns of associations between local/foreign MHC haplotypes and local/foreign parasites. This also indicates that MHC diversity can be preserved through fluctuating selection where different allelic variants are maintained due to spatial variation in parasites. Strong selection against migrants, too often overlooked, can represent a primary reproductive barrier as the consequences of local adaptation can be drastic (Hendry 2004; Nosil 2004). One of these consequences is that selection against migrants can affect and potentially even prevent selection on post-dispersal reproductive barriers and the evolution of conspecific mate preference.

Further analyses should include comprehensive studies of the contribution of each barrier to attempt to reconstruct which barriers evolved when and the conditions under which reproductive barriers evolve in the case of parasite-mediated ecological speciation. Another related question remaining to be answered in this context is under which conditions parasites can drive reproductive isolation and cause speciation (Eizaguirre & Lenz 2010; Karvonen & Seehausen 2012). Indeed, differences in parasite communities between populations do not necessarily lead to differences in MHC pools even if MHC diversity and parasite load correlate (Tobler et al. 2014). To understand the condition under which parasites promote or prevent speciation, it is capital to acknowledge the strength of parasite-mediated selection and the specificity of host-parasite interactions. To answer this question, it is necessary to generalize these findings in other systems and examine genotypic and phenotypic variation associated with reproductive barriers along a continuum of speciation (Feulner *et al.* 2015). This will provide a complete understanding of the general conditions under which parasites can drive ecological speciation.

In the third chapter of my thesis I tested and successfully demonstrated the existence of sperm-mediated paternal effects of parasite infection. This study exemplifies the role of transgenerational effects of paternal infection on offspring life-history and phenotype. Although experimental paternal infection led to high costs, these costs could be compensated by an increased parasite tolerance in the surviving offspring. These results imply that, beyond the role of genes, non-genetic mechanisms can also play a role in host-parasite interactions at ecological time scales. Importantly, these short-time effects can also affect evolutionary dynamics and buffer selection at the genetic level (Mostowy 2012; Tidbury *et al.* 2012). For example, paternal effects granting heightened parasite tolerance to infected offspring will increase the fitness of these primed individuals, even if they are bearing suboptimal immune genotypes. One consequence of such a scenario is the preservation of neutral alleles or alleles associated with susceptibility to parasites at the population level and the maintenance of immunogenetic diversity. As immunogenetic diversity is of major importance for processes in natural and sexual selection (Wegner *et al.* 2003a; Milinski 2006) and even in speciation (Eizaguirre *et al.* 2009a), it is capital to understand how and when parental effects evolve and affect phenotypic and genetic variation in host defenses. I would like to emphasize that this logic is not restricted to host-parasite interactions as acknowledging the relative share of non-genetic transgenerational effects in adaptive evolution provides an exciting addition to the current paradigm in evolutionary theory (e.g. Helanterä & Uller 2010; Klironomos *et al.* 2013b). Further studies should therefore expand this aspect theoretically and empirically, for example by developing mathematical models and experiments exploring the role of non-genetic effects in the maintenance of (immuno)genetic diversity at the population and metapopulation level.

When experimentally infected, males could trigger increased tolerance in their offspring but resistance was mostly genetically determined. This is particularly interesting as resistance is likely to be more pathogen-specific than tolerance which regulates damage control in the host. Interestingly, the concept of tolerance does not necessarily fit in the classic perspective on antagonistic coevolution between host and parasite. This is because increased host tolerance does not necessarily have deleterious effects on parasite fitness. From a historical perspective, theories on host-parasite coevolution assume only the reciprocal evolution of host resistance and parasite virulence (e.g. Paterson *et al.* 2010). Therefore, incorporating the concept of tolerance - as well as causes and consequences of such behavior both for host and parasite

fitness holds a promising future (see Vale *et al.* 2011; Best *et al.* 2014). Noteworthy is that the outcome of evolution of tolerance on parasite virulence is still extensively debated by some authors suggesting that it can lead to a state of "apparent commensalism", multiple stable states or to higher virulence or transmission (Vale 2013). The mechanisms underlying sperm-mediated paternal effects are yet unknown and further studies are also needed here. It would be particularly interesting to understand if epigenetic marks carried across generations lead to context-dependent changes in the offspring's expression of genes involved in damage control of parasitic infection.

Overall, my doctoral research aspired to improve our understanding of the role of parasites in promoting and maintaining diversity in their hosts. To tackle questions attached to this very large and complex problem, I undertook a multivariate approach considering different timepoints in the life history of the stickleback. I also procured a comprehensive view on how parasites can affect traits not only within but also across generations. I illuminated the role of parasites in maintaining genetic and phenotypic diversity, from the level of the sperm phenotype to the population level.

To conclude, the presented work supports the strong role that parasites play in driving host phenotypic and genetic polymorphism and potentially even speciation. I particularly showed that with a deeper understanding of the role of tolerance and non-genetic transmission on host-parasite interactions our theoretical understanding of host-parasite coevolution can and will expand.

Acknowledgments

First of all, I would like to thank Prof. Manfred Milinski for the opportunity to work in his department and for his strong support during the long and many years of my PhD. I feel I learned a lot from your experience and your vast knowledge.

I want to thank my direct supervisors, Christophe “Chris” Eizaguirre and Tobias “Tobi” Lenz, for your unequivocal and unlimited patience. Thanks for teaching me so much during your long time commitment into the supervision of my thesis. Having dynamic supervisors with sometimes opposing views was certainly productive and surely helped me develop my own ideas, as well as my German pragmatism and French enthusiasm. I would like to express my highest respect for Chris who successfully manages to be a brilliant scientist and still give 101% to his students. I would like to express my most profound gratitude to Tobi for having the capacity to teach me so much while calming me down. Awesome!

Martin, I want to thank you for your immeasurable help and mentoring and for showing me the wonderful world of parasites with all the hooks, cysts and complex life histories. I always enjoyed our chats and the knowledge I gained from it.

There have been some friends and colleagues that passed by Plön since I worked there and whom I would like to thank for both scientific discussion and fun times. I want to express my gratitude to the entire “semi-senior” scientist staff that showed me so many sides of how to make good science. The variety of your expertise and the diversity of your experiences made my time in Plön so great. Thank you Benno, Chaitanya, Dan, Demetra, Emilie, Freddy, Irene, Lutz, Kenyon, Mahesh, Philine, Tina and the others I might have forgotten.

I want to express my gras-attitude to Noemie aka the No for all the shared experiences and adventures in the lab, the office, the corridor and the OTS (sic). You are a not only my co-PhD but a true reliable friend. Viva El Copepodo!

The MPI is not only a great place to work but also a community. And I am deeply in debt to this community for my survival in remote Plön. You could always rely on Chai for evening discussion and movies of varying quality, the Colombians for a bit of class and the Germans

and Brits for exciting chats and BBQs. Special thanks to Jens who managed to drag me out of Plön for a year and to my office mates who tolerated me!

I also want to thank my friends from out-of-Plön, for keeping in touch despite the distance: Flo, Chloé, Rachel, Cam, Bea, Julien, Allan, Karo et Melissa. Je voudrais aussi remercier ma famille pour avoir fait l'effort de venir me visiter à Plön et Kiel : Merci Vouille, Giz, Th, Ax, le Petit, Mémé et Pépé.

A high-class thank you Anne for your unreserved and heartwarming support!

To Anja, Britta, Daniel, Derk, Gerhard, Gisela, Helga, Ines, Kerstin, Michael, Nina, Regina, Siggie (Abend!), Steffi, Sybille and ~~ros~~Withe: I put you at the end because you are very important and because none of the crazy experiments would have worked correctly if you would not have been there. It is not only that, and not only that your presence in the corridor balanced the nerdiness of the scientists with the lovely family-feeling of hard working coworkers. All kind of aunts (even you Michael)! Danke!

Bibliography

1. Alcaide M, Rodríguez A, Negro JJ, Serrano D (2012) Male transmission ratio distortion supports MHC-linked cryptic female choice in the lesser kestrel (Aves : Falconidae). *Behavioral Ecology and Sociobiology*, 1467–1473.

2. Anderson R, May R (1982) Coevolution of hosts and parasites. *Parasitology*, **85**, 411–426.

3. Apanius V, Penn D, Slev PPR, Ruff LR, Potts WK (1997) The nature of selection on the major histocompatibility complex. *Critical Reviews in Immunology*, **17**, 179–224.

4. Arnegard ME, McGee MD, Matthews B *et al.* (2014) Genetics of ecological divergence during speciation. *Nature*, **511**, 307–311.

5. Ayres JS, Schneider DS (2012) Tolerance of infections. *Annual review of immunology*, **30**, 271–94.

6. Babik W, Pabijan M, Radwan J (2008) Contrasting patterns of variation in MHC loci in the Alpine newt. *Molecular ecology*, **17**, 2339–55.

7. Bakker TCM, Zbinden M, Frommen JG, Weiss A, Largiadèr CR (2006) Slow fertilization of stickleback eggs: the result of sexual conflict? *BMC ecology*, **6**, 1–8.

8. Barber I (2013) Sticklebacks as model hosts in ecological and evolutionary parasitology. *Trends in Parasitology*, **29**, 556–566.

9. Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature*, **439**, 719–23.

10. Becks L, Agrawal AF (2012) The evolution of sex is favoured during adaptation to new environments. *PLoS biology*, **10**, e1001317.

11. Belloni V, Faivre B, Guerreiro R, Arnoux E, Sorci G (2010) Suppressing an anti-inflammatory cytokine reveals a strong age-dependent survival cost in mice. *PloS one*, **5**, 2–8.

12. Bernardo J (1996) Maternal effects in animal ecology. *American Zoologist*, **105**, 83–105.

13. Bernatchez L, Landry C (2003) MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? *Journal of evolutionary biology*, **16**, 363–77.

14. Berner D, Adams DC, Grandchamp A-C, Hendry AP (2008) Natural selection drives patterns of lake-stream divergence in stickleback foraging morphology. *Journal of evolutionary biology*, **21**, 1653–65.

15. Berner D, Kaeuffer R, Grandchamp A-C *et al.* (2011) Quantitative genetic inheritance of morphological divergence in a lake-stream stickleback ecotype pair: implications for reproductive isolation. *Journal of evolutionary biology*, **24**, 1975–83.

16. Best A, White A, Boots M (2014) The coevolutionary implications of host tolerance. *Evolution*, **68**, 1426–1435.

17. Birkhead TR, Brillard J-P (2007) Reproductive isolation in birds: postcopulatory prezygotic barriers. *Trends in ecology & evolution*, **22**, 266–72.

18. Blais J, Rico C, van Oosterhout C *et al.* (2007) MHC adaptive divergence between closely related and sympatric African cichlids. *PloS one*, **2**, e734.

19. Bonduriansky R (2012) Rethinking heredity, again. *Trends in ecology & evolution*, **27**, 330–336.

20. Bonduriansky R, Day T (2009) Nongenetic Inheritance and Its Evolutionary Implications. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 103–125.

21. Bonneaud C, Gonzalez G, Haussy C *et al.* (2003) Assessing the Cost of Mounting an Immune Response. *The American naturalist*, **161**, 367–379.

22. Bonneaud C, Pérez-Tris J, Federici P, Chastel O, Sorci G (2006) Major histocompatibility alleles associated with local resistance to malaria in a passerine. *Evolution*, **60**, 383–9.

23. Borghans JA, Beltman JB, De Boer RJ (2004) MHC polymorphism under host-pathogen coevolution. *Immunogenetics*, **55**, 732–9.

24. Boughman JW (2001) Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, **411**, 944–8.

25. Boughman JW (2013) Ecological speciation: Selection and the origin of species. *Current Zoology*, **59**, 1–7.

26. Boughman JW, Rundle HD, Schluter D (2005) Parallel evolution of sexual isolation in sticklebacks. *Evolution*, **59**, 361–73.

27. Breckels RD, Neff BD (2014) Rapid evolution of sperm length in response to increased temperature in an ectothermic fish. *Evolutionary Ecology*, **28**, 521–533.

28. Bromfield JJ, Schjenken JE, Chin PY *et al.* (2014) Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *Proceedings of the National Academy of Sciences*, **111**, 2200–2205.

29. Buechler K, Fitze P, Gottstein B, Jacot A, Richner H (2002) Parasite-induced maternal response in a natural bird population. *Journal of Animal Ecology*, **71**, 247–252.

30. Burgess SC, Marshall DJ (2014) Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos*, **123**, 769–776.

31. Butlin R, Debelle A, Kerth C *et al.* (2012) What do we need to know about speciation? *Trends in ecology & evolution*, **27**, 27–39.

32. Butlin RK, Galindo J, Grahame JW (2008) Review. Sympatric, parapatric or allopatric: the most important way to classify speciation? *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **363**, 2997–3007.

33. Chain FJJ, Feulner PGD, Panchal M *et al.* (2014) Extensive Copy-Number Variation of Young Genes across Stickleback Populations. *PLoS genetics*, **10**, e1004830.

34. Chargé R, Saint Jalme M, Lacroix F, Cadet A, Sorci G (2010) Male health status, signalled by courtship display, reveals ejaculate quality and hatching success in a lekking species. *The Journal of animal ecology*, **79**, 843–50.

35. Chellappa S, Huntingford FA, Strang RHC, Thomson RY (1995) Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *Journal of Fish Biology*, **47**, 775–787.

36. Clarke K (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

37. Clarke B, Kirby D (1966) Maintenance of histocompatibility polymorphisms. *Nature*, **211**, 999–1000.

38. Cohen J (1988) *Statistical power analysis for the behavioral sciences*. Routledge Academic.

39. Colosimo PFP, Hosemann KKE, Balabhadra S *et al.* (2005) Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. *Science*, **307**, 1928–33.

40. Coyne JA, Orr. HA (2004) *Speciation* (U Sunderland, MA, Ed,). Sinauer Associates.

41. Crean AJ, Bonduriansky R (2014) What is a paternal effect? *Trends in Ecology & Evolution*, **29**, 554–559.

42. Crean AJ, Dwyer JM, Marshall DJ (2012) Fertilization Is Not a New Beginning: The Relationship between Sperm Longevity and Offspring Performance. *PloS one*, **7**, 1–6.

43. Crean AJ, Dwyer JM, Marshall DJ (2013) Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. *Ecology*, **94**, 2575–2582.

44. Crespi B, Nosil P (2013) Conflictual speciation: species formation via genomic conflict. *Trends in Ecology & Evolution*, **28**, 48–57.

45. Curley J, Mashoodh R, Champagne F (2011) Epigenetics and the origins of paternal effects. *Hormones and behavior*, **59**, 306–314.

46. Danchin É, Charmantier A (2011) Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. *Nature Reviews Genetics*, **12**, 475–486.

47. Darwin C (1859) *The origin of species by means of natural selection: or, the preservation of favored races in the struggle for life*. John Murray.

48. Darwin C (1871) *The Descent of Man, and Selection in Relation to Sex*. John Murray.

49. Day T, Bonduriansky R (2011) A unified approach to the evolutionary consequences of genetic and nongenetic inheritance. *The American naturalist*, **178**, E18–36.

50. Decaestecker E, Gaba S, Raeymaekers JAM *et al.* (2007) Host-parasite "Red Queen" dynamics archived in pond sediment. *Nature*, **450**, 870–3.

51. Dionne M, Miller KM, Dodson JJ, Bernatchez L (2009) MHC standing genetic variation and pathogen resistance in wild Atlantic salmon. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 1555–65.

52. Dobzhansky T (1940) Speciation as a stage in evolutionary divergence. *American Naturalist*, **74**, 312–321.

53. Doherty PC, Zinkernagel RM (1975) Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature*, **256**, 50–52.

54. Dybdahl M, Lively C (1998) Host-parasite coevolution: evidence for rare advantage and time-lagged selection in a natural population. *Evolution*, **52**, 1057–1066.

55. Eady PPE (2001) Postcopulatory, prezygotic reproductive isolation. *Journal of Zoology*, **253**, 47–52.

56. Ebert D, Hamilton WWD (1996) Sex against virulence : the coevolution of parasitic diseases. *Trends in Ecology & Evolution*, **11**, 79–82.

57. Edwards S V, Hedrick PW (1998) Evolution and ecology of MHC molecules: from genomics to sexual selection. *Trends in ecology & evolution*, **13**, 305–11.

58. Eggert H (2014) Different effects of paternal trans-generational immune priming on survival and immunity in step and genetic offspring. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 1–8.

59. Ehrlich P (1892) Über immunität durch Vererbung und Säungung. *Zeitschrift fur Hygiene und Infektionskrankheiten*, **12**, 183–203.

60. Eizaguirre C, Lenz TL (2010) Major histocompitability complex polymorphism: dynamics and consequences of parasite-mediated local adaptation in fishes. *Journal of Fish Biology*, **77**, 2023–2047.

61. Eizaguirre C, Lenz TL, Kalbe M, Milinski M (2012a) Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nature Communications*, **3**, 621–626.

62. Eizaguirre C, Lenz TL, Kalbe M, Milinski M (2012b) Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. *Ecology letters*, **15**, 723–731.

63. Eizaguirre C, Lenz TL, Sommerfeld RD *et al.* (2011) Parasite diversity, patterns of MHC II variation and olfactory based mate choice in diverging three-spined stickleback ecotypes. *Evolutionary Ecology*, **25**, 605–622.

64. Eizaguirre C, Lenz TL, Traulsen A, Milinski M (2009a) Speciation accelerated and stabilized by pleiotropic major histocompatibility complex immunogenes. *Ecology letters*, **12**, 5–12.

65. Eizaguirre C, Yeates SE, Lenz TL, Kalbe M, Milinski M (2009b) MHC-based mate choice combines good genes and maintenance of MHC polymorphism. *Molecular ecology*, **18**, 3316–29.

66. Elofsson H, Van Look KJW, Sundell K, Sundh H, Borg B (2006) Stickleback sperm saved by salt in ovarian fluid. *The Journal of experimental biology*, **209**, 4230–7.

67. Elofsson H, Mcallister BG, Kime DE, Mayer I, Borg B (2003) Long lasting stickleback sperm; is ovarian fluid a key to success in fresh water? *Journal of Fish Biology*, **63**, 240–253.

68. Feulner PG, Chain FJ, Panchal M *et al.* (2015) Genomics of Divergence along a Continuum of Incipient Ecological Speciation. *PLoS Genetics*, **in press**.

69. Firman RC, Simmons LW (2014) No Evidence of Conpopulation Sperm Precedence between Allopatric Populations of House Mice. *PloS one*, **9**, e107472.

70. Fisher RA (1930) *The Genetical Theory of Natural Selection*.

71. Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*, **139**, 603–622.

72. Fritz R, Moulia C, Newcombe G (1999) Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. *Annual Review of Ecology and Systematics*, **30**, 565–91.

73. Gallizzi K, Alloitteau O, Harrang E, Richner H (2008) Fleas, parental care, and transgenerational effects on tick load in the great tit. *Behavioral Ecology*, **19**, 1225–1234.

74. Gandon S, Buckling A, Decaestecker E, Day T (2008) Host-parasite coevolution and patterns of adaptation across time and space. *Journal of evolutionary biology*, **21**, 1861–6.

75. Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology*, **21**, 434–443.

76. Gavrilets S (2003) Perspective: models of speciation: what have we learned in 40 years? *Evolution*, **57**, 2197–215.

77. Geyer LB, Palumbi SR (2005) Conspecific sperm precedence in two species of tropical sea urchins. *Evolution*, **59**, 97–105.

78. Gimenez-Bonafe P (2000) Characterization and evolutionary relevance of the sperm nuclear basic proteins from stickleback fish. *Molecular reproduction and development*, **193**.

79. Goldberg AD, Allis CD, Bernstein E (2007) Epigenetics: a landscape takes shape. *Cell*, **128**, 635–8.

80. Gould SJ, Lewontin RC (1979) The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme. *Proceedings of the Royal Society B: Biological Sciences*, **205**, 581–598.

81. Gower J, Legendre P (1986) Metric and Euclidean properties of dissimilarity coefficients. *Journal of classification*, **48**, 5–48.

82. Graham AL, Shuker DM, Pollitt LC *et al.* (2011) Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. *Functional Ecology*, **25**, 5–17.

83. Griffiths R (2000) DNA sex identification in the three-spined stickleback. *Journal of Fish Biology*, **57**, 1331–1334.

84. Grindstaff JL, Brodie ED, Ketterson ED (2003) Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings. Biological sciences / The Royal Society*, **270**, 2309–19.

85. Haldane J (1992) Disease and evolution. *Current Science*, **63**, 599–603.

86. Hamilton W (1980) Sex versus non-sex versus parasite. *Oikos*, **35**, 282–290.

87. Hamilton WD, Axelrod R, Tanese R (1990) Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 3566–73.

88. Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? *Science*, **218**, 384–387.

89. Hasselquist D, Nilsson J-A (2009) Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 51–60.

90. Hawthorne D, Via S (2001) Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature*, **412**, 28–31.

91. Hayward LS, Wingfield JC (2004) Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *General and Comparative Endocrinology*, **135**, 365–371.

92. Hedrick PW (2002) Pathogen resistance and genetic variation at MHC loci. *Evolution*, **56**, 1902–8.

93. Heins DC, Baker JA (2008) The stickleback Schistocephalus host parasite system as a model for understanding the effect of a macroparasite on host reproduction. *Behaviour*, **145**, 625–645.

94. Helanterä H, Uller T (2010) The Price equation and extended inheritance. *Philosophy & Theory in Biology*, **2**, pp.

95. Hendry AP (2004) Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evolutionary Ecology Research*, **6**, 1219–1236.

96. Hendry AP (2009) Ecological speciation! Or the lack thereof? *Canadian Journal of Fisheries and Aquatic Sciences*, **66**, 1383–1398.

97. Hendry AP, Bolnick DI, Berner D, Peichel CL (2009) Along the speciation continuum in sticklebacks. *Journal of fish biology*, **75**, 2000–36.

98. Henrich T, Benesh DP, Kalbe M (2013) Hybridization between two cestode species and its consequences for intermediate host range. *Parasites & vectors*, **6**, 33.

99. Hewitt G, Mason P, Nichols R (1989) Sperm precedence and homogamy across a hybrid zone in the alpine grasshopper Podisma pedestris. *Heredity*, **62**, 343–353.

100. Hill A (1991) HLA associations with malaria in Africa: some implications for MHC evolution. In: *Molecular evolution of the major histocompatibility complex* (eds Klein J, Klein D), pp. 404–420.

101. Hill AVS (1998) The immunogenetics of human infectious diseases. *Annual review of immunology*, **16**, 593–617.

102. Howard D (1999) Conspecific sperm and pollen precedence and speciation. *Annual Review of Ecology and Systematics*, **30**, 109–132.

103. Immler S, Hamilton MB, Poslusny NJ, Birkhead TR, Epifanio JM (2011) Post-mating reproductive barriers in two unidirectionally hybridizing sunfish (Centrarchidae: Lepomis). *Journal of evolutionary biology*, **24**, 111–20.

104. Jablonka E (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Quarterly Review of Biology*, **84**, 131–176.

105. Jablonka E, Lamb M (1995) *Epigenetic inheritance and evolution - The Lamarckian dimension*. Oxford University Press, USA.

106. Jaenike J (1978) An hypothesis to account for the maintenance of sex within populations. *Evol. theory*, **3**, 191–194.

107. Jäger I, Eizaguirre C, Griffiths SW *et al.* (2007) Individual MHC class I and MHC class IIB diversities are associated with male and female reproductive traits in the three-spined stickleback. *Journal of evolutionary biology*, **20**, 2005–15.

108. Jakob E, Marshall S, Uetz G (1996) Estimating fitness: a comparison of body condition indices. *Oikos*, **77**, 61–67.

109. Jakobsen J (2011) Parasite communities of two three-spined stickleback (Gasterosteus aculeatus) populations - Effects of a local-scale host introduction? Master thesis, University of Tromsø.

110. Janeway CA (2001) How the immune system protects the host from infection. *Microbes and infection / Institut Pasteur*, **3**, 1167–71.

111. Janeway CA, Travers P, Walport M, Capra JD (2001) *Immunobiology: the immune system in health and disease*. Churchill Livingstone.

112. Jiang L, Zhang JJ, Wang J *et al.* (2013) Sperm , but Not Oocyte , DNA Methylome Is Inherited by Zebrafish Early Embryos. *Cell*, **153**, 773–784.

113. Jokela J, Dybdahl MF, Lively CM (2009) The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. *The American naturalist*, **174 Suppl** , S43–53.

114. Jones FC, Grabherr MG, Chan YF *et al.* (2012) The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*, **484**, 55–61.

115. Kalbe M, Eizaguirre C, Dankert I *et al.* (2009) Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 925–34.

116. Kalbe M, Kurtz J (2006) Local differences in immunocompetence reflect resistance of sticklebacks against the eye fluke Diplostomum pseudospathaceum. *Parasitology*, **132**, 105–16.

117. Kalbe M, Wegner KM, Reusch T (2002) Dispersion patterns of parasites in 0+ year three-spined sticklebacks: a cross population comparison. *Journal of Fish Biology*, **60**, 1529–1542.

118. Kalinowski S, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular ecology*, **16**, 1099–1106.

119. Kaltz O, Shykoff JA (1998) Local adaptation in host–parasite systems. *Heredity*, **81**, 361–370.

120. Kamiya T, O'Dwyer K, Westerdahl H, Senior A, Nakagawa S (2014) A quantitative review of MHC-based mating preference: the role of diversity and dissimilarity. *Molecular Ecology*, **23**, 5151–5163.

121. Kappeler L, Meaney MJM (2010) Epigenetics and parental effects. *Bioessays*, **32**, 818–27.

122. Karvonen A, Seehausen O (2012) The Role of Parasitism in Adaptive Radiations—When Might Parasites Promote and When Might They Constrain Ecological Speciation? *International Journal of Ecology*, **2012**, 1–20.

123. Kaufmann J, Lenz TL, Milinski M, Eizaguirre C (2014) Experimental parasite infection reveals costs and benefits of paternal effects (D Marshall, Ed,). *Ecology Letters*, **17**, 1409–1417.

124. Kawecki TJ (1998) Red Queen Meets Santa Rosalia : Arms Races and the Evolution of Host Specialization. *The American naturalist*, **152**, 635–651.

125. Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225–1241.

126. Kekäläinen J, Pirhonen J, Taskinen J (2014) Do highly ornamented and less parasitized males have high quality sperm? - an experimental test for parasite-induced reproductive trade-offs in European minnow (Phoxinus phoxinus). *Ecology and Evolution*, **4**, 4237–4245.

127. Kelley J, Walter L, Trowsdale J (2005) Comparative genomics of major histocompatibility complexes. *Immunogenetics*, **56**, 683–95.

128. Kime D, Ebrahimi M, Nysten K *et al.* (1996) Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish; application to the effects of heavy metals. *Aquatic toxicology*, **36**, 223–237.

129. Kime DE, Van Look KJ, McAllister BG *et al.* (2001) Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish. *Comparative biochemistry and physiology. Toxicology & pharmacology*, **130**, 425–33.

130. King KC, Jokela J, Lively CM (2011) Trematode parasites infect or die in snail hosts. *Biology letters*, **7**, 265–268.

131. Klironomos FFD, Berg J, Collins S (2013a) How epigenetic mutations can affect genetic evolution : Model and mechanism. *BioEssays : news and reviews in molecular, cellular and developmental biology*, **35**, 571–578.

132. Klironomos FD, Berg J, Collins S (2013b) How epigenetic mutations can affect genetic evolution : Model and mechanism. *BioEssays : news and reviews in molecular, cellular and developmental biology*, **35**, 571–578.

133. Konijnendijk N, Raeymaekers JAM, Vandeuren S, Jacquemin L, Volckaert FAM (2013) Testing for local adaptation in the Gasterosteus-Gyrodactylus host-parasite system. *Evolutionary Ecology Research*, **15**, 489–502.

134. Koskella B, Lively CM (2009) Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. *Evolution*, **63**, 2213–21.

135. Krobbach CK, Kalbe M, Kurtz J, Scharsack JP (2007) Infectivity of two nematode parasites, Camallanus lacustris and Anguillicola crassus, in a paratenic host, the three-spined stickleback Gasterosteus aculeatus. *Diseases of aquatic organisms*, **74**, 119–26.

136. Kronforst MR, Young LG, Kapan DD *et al.* (2006) Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 6575–80.

137. Kuris AM, Hechinger RF, Shaw JC *et al.* (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature*, **454**, 515–8.

138. Kurtz J, Franz K (2003) Evidence for memory in invertebrate immunity. *Nature*, **425**, 37–38.

139. Kurtz J, Kalbe M, Langefors A *et al.* (2007) An experimental test of the immunocompetence handicap hypothesis in a teleost fish: 11-ketotestosterone suppresses innate immunity in three-spined sticklebacks. *The American naturalist*, **170**, 509–19.

140. Lajeunesse MJ, Forbes MR (2002) Host range and local parasite adaptation. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 703–710.

141. Laland KN, Odling-Smee J, Myles S (2010) How culture shaped the human genome: bringing genetics and the human sciences together. *Nature Reviews Genetics*, **11**, 137–48.

142. Landry C, Bernatchez L (2001) Comparative analysis of population structure across environments and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic salmon (Salmo salar). *Molecular ecology*, **10**, 2525–39.

143. Lefebvre F, Mounaix B (2004) Impacts of the swimbladder nematode Anguillicola crassus on Anguilla anguilla: variations in liver and spleen masses. *Journal of fish biology*, **33**, 435–447.

144. Lemke H, Coutinho A, Lange H (2004) Lamarckian inheritance by somatically acquired maternal IgG phenotypes. *Trends in immunology*, **25**, 180–6.

145. Lenz TL (2011) Computational prediction of MHC II-antigen binding supports divergent allele advantage and explains trans-species polymorphism. *Evolution*, **65**, 2380–90.

146. Lenz TL, Eizaguirre C, Becker S, Reusch TBH (2009a) RSCA genotyping of MHC for high-throughput evolutionary studies in the model organism three-spined stickleback Gasterosteus aculeatus. *BMC evolutionary biology*, **9**, 1–13.

147. Lenz TL, Eizaguirre C, Rotter B, Kalbe M, Milinski M (2013) Exploring local immunological adaptation of two stickleback ecotypes by experimental infection and transcriptome-wide digital gene expression analysis. *Molecular ecology*, **22**, 774–86.

148. Lenz TL, Eizaguirre C, Scharsack JP, Kalbe M, Milinski M (2009b) Disentangling the role of MHC-dependent “good genes” and “compatible genes” in mate-choice decisions of three-spined sticklebacks Gasterosteus aculeatus under semi-natural conditions. *Journal of fish biology*, **75**, 2122–42.

149. De León LF, Bermingham E, Podos J, Hendry AP (2010) Divergence with gene flow as facilitated by ecological differences: within-island variation in Darwin’s finches. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **365**, 1041–52.

150. Liljedal S, Folstad I, Skarstein F (1999) Secondary sex traits, parasites, immunity and ejaculate quality in the Arctic charr. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 1893–98.

151. Linder JE, Promislow D (2009) Cross-generational fitness effects of infection in Drosophila melanogaster. *Fly*, **3**, 143–150.

152. Little T (2002) The evolutionary significance of parasitism: do parasite-driven genetic dynamics occur ex silico? *Journal of Evolutionary Biology*, **15**, 1–9.

153. Lively CM (1987) Evidence from a new zealand snail for the maintenance of sex by parasitism. *Nature*, **328**, 519–521.

154. Lively CM (1999) Migration, Virulence, and the Geographic Mosaic of Adaptation by Parasites. *The American Naturalist*, **153**, S34–S47.

155. Lively CM, Dybdahl MF (2000) Parasite adaptation to locally common host genotypes. *Nature*, **405**, 679–81.

156. Long GH, Chan BHK, Allen JE, Read AF, Graham AL (2008a) Experimental manipulation of immune-mediated disease and its fitness costs for rodent malaria parasites. *BMC evolutionary biology*, **8**, 1–11.

157. Long GH, Chan BHK, Allen JE, Read AF, Graham AL (2008b) Blockade of TNF receptor 1 reduces disease severity but increases parasite transmission during Plasmodium chabaudi chabaudi infection. *International journal for parasitology*, **38**, 1073–81.

158. Lorch PD, Servedio MR (2007) The evolution of conspecific gamete precedence and its effect on reinforcement. *Journal of evolutionary biology*, **20**, 937–949.

159. Lorch PD, Servedio MR, Url S (2011) Postmating-Prezygotic Isolation Is Not an Important Source of Selection for Reinforcement within and between Species in Drosophila pseudoobscura and D . persimilis. *Evolution*, **59**, 1039–1045.

160. Losdat S, Richner H, Blount JD, Helfenstein F (2011) Immune Activation Reduces Sperm Quality in the Great Tit. *PLoS One*, **6**, 1–10.

161. Losos J, Mahler D (2010) Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. In: *Evolution since Darwin: the First 150 Years* (eds Bell MA, Futuyma DJ, Eanes WF, Levinton MA), pp. 381–420. Sinauer Assoc.: Sunderland, MA.

162. Losos JB, Ricklefs RE (2009) Adaptation and diversification on islands. *Nature*, **457**, 830–6.

163. Løvlie H, Gillingham MAF, Worley K *et al.* (2013) Cryptic female choice favours sperm from major histocompatibility complex-dissimilar males. … *of the Royal* ….

164. Ludlow a M, Magurran a E (2006) Gametic isolation in guppies (Poecilia reticulata). *Proceedings. Biological sciences / The Royal Society*, **273**, 2477–82.

165. Maan ME (2006) Fitness correlates of male coloration in a Lake Victoria cichlid fish. *Behavioral Ecology*, **17**, 691–699.

166. Maan ME, Van Rooijen AMC, Van Alphen JJM, Seehausen O (2008) Parasite-mediated sexual selection and species divergence in Lake Victoria cichlid fish. *Biological Journal of the Linnean Society*, **94**, 53–60.

167. Maan ME, Seehausen O (2011) Ecology, sexual selection and speciation. *Ecology letters*, **14**, 591–602.

168. MacColl ADC (2009) Parasite burdens differ between sympatric three-spined stickleback species. *Ecography*, **32**, 153–160.

169. MacColl ADC, Chapman S (2010) Parasites can cause selection against migrants following dispersal between environments. *Functional ecology*, **24**, 847–856.

170. MacDougall-Shackleton E, Derryberry E, Hahn T (2002) Nonlocal male mountain white-crowned sparrows have lower paternity and higher parasite loads than males singing local dialect. *Behavioral Ecology*, **13**, 682–689.

171. Macnab V, Katsiadaki I, Barber I (2009) Reproductive potential of Schistocephalus solidus-infected male three-spined stickleback Gasterosteus aculeatus from two U.K. populations. *Journal of fish biology*, **75**, 2095–107.

172. Malo D, Skamene E (1994) Genetic control of host resistance to infection. *Trends in genetics*, **10**, 365–71.

173. Marshall DJ (2008) Transgenerational Plasticity in the Sea : Context-Dependent Maternal Effects Across the Life History. *Ecology*, **89**, 418–427.

174. Marshall DJ, Uller T (2007) When is a maternal effect adaptive? *Oikos*, **116**, 1957–1963.

175. Martín-Coello J, Benavent-Corai J, Roldan ERS, Gomendio M (2009) Sperm competition promotes asymmetries in reproductive barriers between closely related species. *Evolution*, **63**, 613–23.

176. Matthews B, Harmon LJ, M'Gonigle L, Marchinko KB, Schaschl H (2010) Sympatric and Allopatric Divergence of MHC Genes in Threespine Stickleback (J Demuth, Ed,). *PLoS ONE*, **5**, e10948.

177. Maynard Smith J (1978) *The Evolution of Sex*. Cambridge Univ. Press, Cambridge, UK.

178. Mayr E (1942) *Systematics and the Origin of Species*.

179. Mayr E (1947) Ecological Factors in Speciation. *Evolution*, **1**, 263–288.

180. Mayr E (1963) *Animal species and evolution*. Harvard University Press; London: Oxford University Press.

181. McCairns RJS, Bourget S, Bernatchez L (2011) Putative causes and consequences of MHC variation within and between locally adapted stickleback demes. *Molecular ecology*, **20**, 486–502.

182. McGlothlin JW, Galloway LF (2013) The Contribution of Maternal Effects To Selection Response: an Empirical Test of Competing Models. *Evolution*, **68**, 549–558.

183. McKinnon JS, Mori S, Blackman BK *et al.* (2004) Evidence for ecology's role in speciation. *Nature*, **429**, 294–298.

184. McKinnon JS, Rundle HD (2002) Speciation in nature: the threespine stickleback model systems. *Trends in Ecology & Evolution*, **17**, 480–488.

185. Medzhitov R, Schneider DS, Soares MP (2012) Disease tolerance as a defense strategy. *Science*, **335**, 936–41.

186. Mendelson TC, Imhoff VE, Venditti JJ (2007) The accumulation of reproductive barriers during speciation: postmating barriers in two behaviorally isolated species of darters (Percidae: Etheostoma). *Evolution*, **61**, 2596–606.

187. Milinski M (1979) An evolutionarily stable Feeding strategy in sticklebacks. *Zeitschrift für Tierpsychologie*, **40**, 36–40.

188. Milinski M (1987) Tit for tat in sticklebacks and the evolution of cooperation. *Nature*, **327**, 433–435.

189. Milinski M (2006) The Major Histocompatibility Complex, Sexual Selection, and Mate Choice. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 159–186.

190. Milinski M, Bakker TTCM (1990) Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, **344**, 330–333.

191. Milinski M, Griffiths S, Wegner KM *et al.* (2005) Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 4414–8.

192. Miller MR, White A, Boots M (2005) The evolution of host resistance: tolerance and control as distinct strategies. *Journal of theoretical biology*, **236**, 198–207.

193. Moller A, Christe P, Lux E (1999) Parasitism, host immune function, and sexual selection. *Quarterly Review of Biology*, **74**, 3–20.

194. Moore J (2002) *Parasites and the behavior of animals*. Oxford University Press, USA.

195. Morgan H, Sutherland H, Martin D, Whitelaw E (1999) Epigenetic inheritance at the agouti locus in the mouse. *Nature genetics*, **23**, 314–318.

196. Moritz C (1991) Parasite loads in parthenogenetic and sexual lizards (Heteronotia binoei): support for the Red Queen hypothesis. *Proceedings of the Royal Society B: Biological Sciences*, **244**, 145–149.

197. Morran LT, Schmidt OG, Gelarden IA *et al.* (2011) Running with the Red Queen: Host-Parasite Coevolution Selects for Biparental Sex. *Science*, **333**, 216–218.

198. Mostowy R (2012) Non-genetic inheritance and the patterns of antagonistic coevolution. *BMC evolutionary biology*, **12**, 1–10.

199. Mousseau TA, Fox CW (1998) *Maternal effects as adaptations* (TA Mousseau, C Fox, Eds,). Oxford University Press, Oxford, New York.

200. Mousseau TA, Uller T, Wapstra E, Badyaev A V (2009) Evolution of maternal effects: past and present. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 1035–8.

201. Nagy E, Rice K (1997) Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution*, **51**, 1079–1089.

202. Natsopoulou ME, Pálsson S, Ólafsdóttir GÁ (2012) Parasites and parallel divergence of the number of individual MHC alleles between sympatric three-spined stickleback Gasterosteus aculeatus morphs in Iceland. *Journal of fish biology*, **81**, 1696–714.

203. Niemiller ML, Fitzpatrick BM, Miller BT (2008) Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: Gyrinophilus) inferred from gene genealogies. *Molecular ecology*, **17**, 2258–75.

204. Nosil P (2004) Reproductive isolation caused by visual predation on migrants between divergent environments. *Proceedings. Biological sciences / The Royal Society*, **271**, 1521–8.

205. Nosil P, Harmon LJ, Seehausen O (2009) Ecological explanations for (incomplete) speciation. *Trends in ecology & evolution*, **24**, 145–56.

206. Nosil P, Vines TTH, Funk DDJ (2005) Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, **59**, 705–719.

207. Nowak M, Tarczy-Hornoch K, Austyn J (1992) The optimal number of major histocompatibility complex molecules in an individual. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 10896–9.

208. Ortiz-Barrientos D, Grealy A, Nosil P (2009) The genetics and ecology of reinforcement: implications for the evolution of prezygotic isolation in sympatry and beyond. *Annals of the New York Academy of Sciences*, **1168**, 156–82.

209. Palumbi SRSR (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, **25**, 547–572.

210. Paterson S, Vogwill T, Buckling A *et al.* (2010) Antagonistic coevolution accelerates molecular evolution. *Nature*, **464**, 275–8.

211. Peichel CL, Nereng KS, Ohgi K a *et al.* (2001) The genetic architecture of divergence between threespine stickleback species. *Nature*, **414**, 901–5.

212. Peterson DA, Hilborn R, Hauser L (2014) Local adaptation limits lifetime reproductive success of dispersers in a wild salmon metapopulation. *Nature communications*, **5**, 1–7.

213. Piertney SB, Oliver MK (2006) The evolutionary ecology of the major histocompatibility complex. *Heredity*, **96**, 7–21.

214. Pike TW, Blount JD, Lindström J, Metcalfe NB (2010) Dietary carotenoid availability, sexual signalling and functional fertility in sticklebacks. *Biology letters*, **6**, 191–3.

215. Playfair J, Taverne J, Bate C, de Souza J (1990) The malaria vaccine: anti-parasite or anti-disease? *Immunology today*, **11**, 25–7.

216. Postma E, Siitari H, Schwabl H, Richner H, Tschirren B (2014) The multivariate egg: quantifying within- and among-clutch correlations between maternally derived yolk immunoglobulins and yolk androgens using multivariate mixed models. *Oecologia*, **174**, 631–8.

217. Potts WK, Slev PR (1995) Pathogen-based models favoring MHC genetic diversity. *Immunological reviews*, **143**, 181–97.

218. Poulin R (1996) How many parasite species are there: are we close to answers? *International journal for parasitology*, **26**, 1127–9.

219. Poulin R (2006) *Evolutionary Ecology of Parasites*. Princeton University Press, Princeton.

220. Poulin R, Krasnov BR, Mouillot D (2011) Host specificity in phylogenetic and geographic space. *Trends in parasitology*, **27**, 355–361.

221. Poulin R, Thomas F (2008) Epigenetic effects of infection on the phenotype of host offspring: parasites reaching across host generations. *Oikos*, **117**, 331–335.

222. Poullain V, Gandon S, Brockhurst MA, Buckling A, Hochberg ME (2008) The evolution of specificity in evolving and coevolving antagonistic interactions between a bacteria and its phage. *Evolution;*, **62**, 1–11.

223. Price C, Kim C, Posluszny J, Coyne J (2000) Mechanisms of conspecific sperm precedence in Drosophila. *Evolution*, **54**, 2028–2037.

224. Price P, Westoby M, Rice B, Atsatt P (1986) Parasite mediation in ecological interactions. *Annual Review of Ecology and Systematics*, **17**, 487–505.

225. R Development Core Team (2014) R: A language and environment for statistical computing.

226. Råberg L, Graham AL, Read AF (2009) Decomposing health: tolerance and resistance to parasites in animals. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 37–49.

227. Råberg L, Sim D, Read AF (2007) Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science*, **318**, 812–4.

228. Radwan J, Kuduk K, Levy E, LeBas N, Babik W (2014) Parasite load and MHC diversity in undisturbed and agriculturally modified habitats of the ornate dragon lizard. *Molecular ecology*, **23**, 5966–78.

229. Raeymaekers JAM, Boisjoly M, Delaire L *et al.* (2010) Testing for mating isolation between ecotypes: laboratory experiments with lake, stream and hybrid stickleback. *Journal of Evolutionary Biology*, **23**, 2694–708.

230. Raeymaekers JAM, Hablützel PI, Grégoir AF *et al.* (2013) Contrasting parasite communities among allopatric colour morphs of the Lake Tanganyika cichlid Tropheus. *BMC evolutionary biology*, **13**, 1–16.

231. Rafferty NE, Boughman JW (2006) Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. *Behavioral Ecology*, **17**, 965–970.

232. Ramos R, Garnier R, González-Solís J, Boulinier T (2014) Long Antibody Persistence and Transgenerational Transfer of Immunity in a Long-Lived Vertebrate. *The American Naturalist*, **184**, 764–776.

233. Rando OJ (2012) Daddy issues : paternal effects on phenotype. *Cell*, **151**, 702–708.

234. Räsänen K, Hendry AP (2014) Asymmetric reproductive barriers and mosaic reproductive isolation: insights from Misty lake-stream stickleback. *Ecology and evolution*, **4**, 1166–75.

235. Rauch G, Kalbe M, Reusch TBH (2006) Relative importance of MHC and genetic background for parasite load in a field experiment. *Evolutionary Ecology Research*, **8**, 373–386.

236. Reid JM, Arcese P, Keller LF, Hasselquist D (2006) Long-term maternal effect on offspring immune response in song sparrows Melospiza melodia. *Biology letters*, **2**, 573–6.

237. Renaut S, Maillet N, Normandeau E *et al.* (2012) Genome-wide patterns of divergence during speciation: the lake whitefish case study. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **367**, 354–63.

238. Reusch TBH, Schaschl H, Wegner KM (2004) Recent duplication and inter-locus gene conversion in major histocompatibility class II genes in a teleost, the three-spined stickleback. *Immunogenetics*, **56**, 427–37.

239. Reusch T, Wegner KM, Kalbe M (2001) Rapid genetic divergence in postglacial populations of threespine stickleback (Gasterosteus aculeatus): the role of habitat type, drainage and geographical proximity. *Molecular ecology*, **10**, 2435–45.

240. Ricklefs RE (2010) Host-pathogen coevolution, secondary sympatry and species diversification. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **365**, 1139–1147.

241. Ritchie MG (2007) Sexual Selection and Speciation. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 79–102.

242. Robinson J, Halliwell J a, McWilliam H *et al.* (2013) The IMGT/HLA database. *Nucleic acids research*, **41**, D1222–7.

243. Roesti M, Gavrilets S, Hendry AP, Salzburger W, Berner D (2014) The genomic signature of parallel adaptation from shared genetic variation. *Molecular ecology*, **23**, 3944–3956.

244. Rogers SM, Bernatchez L (2007) The genetic architecture of ecological speciation and the association with signatures of selection in natural lake whitefish (Coregonus sp. Salmonidae) species pairs. *Molecular biology and evolution*, **24**, 1423–38.

245. De Roij J, Harris PD, Maccoll ADC (2011) Divergent resistance to a monogenean flatworm among three-spined stickleback populations. *Functional Ecology*, **25**, 217–226.

246. Rosenblum EB, Harmon LJ (2011) "Same same but different": replicated ecological speciation at White Sands. *Evolution*, **65**, 946–60.

247. Roth O, Joop G, Eggert H *et al.* (2010) Paternally derived immune priming for offspring in the red flour beetle, Tribolium castaneum. *The Journal of animal ecology*, **79**, 403–13.

248. Roth O, Klein V, Beemelmanns A, Scharsack JP, Reusch TBH (2012) Male pregnancy and biparental immune priming. *The American Naturalist*, **180**, 802–814.

249. Roy BA, Kirchner JW (2000) Evolutionary dynamics of pathogen resistance and tolerance. *Evolution*, **54**, 51–63.

250. Rundle HD (2002) A test of ecologically dependent postmating isolation between sympatric sticklebacks. *Evolution*, **56**, 322–9.

251. Rundle HD, Nosil P (2005) Ecological speciation. *Ecology Letters*, **8**, 336–352.

252. Rushbrook BJ, Barber I (2006) Nesting, courtship and kidney hypertrophy in Schistocephalus-infected male three-spined stickleback from an upland lake. *Journal of Fish Biology*, **69**, 870–882.

253. Ryan M, Wagner W (1987) Asymmetries in mating preferences between species: female swordtails prefer heterospecific males. *Science*, **236**, 595–597.

254. Sadd BM, Schmid-hempel P (2009) Ecological and evolutionary implications of specific immune responses. In: *Insect infection and immunity: evolution, ecology, and mechanisms* (eds Rolff J, Reynolds SE), pp. 225–240. Oxford University Press; Oxford, New York.

255. Sage R, Heyneman D, Lim K, Wilson A (1986) Wormy mice in a hybrid zone. *Nature*, **324**, 60–63.

256. Saino N, Dall'Ara P (2002) Early maternal effects and antibacterial immune factors in the eggs, nestlings and adults of the barn swallow. *Journal of Evolutionary Biology*, **15**, 735–743.

257. Scharsack JP, Kalbe M (2014) Differences in susceptibility and immune responses of three-spined sticklebacks (Gasterosteus aculeatus) from lake and river ecotypes to sequential infections with the eye fluke Diplostomum pseudospathaceum. *Parasites & vectors*, **7**, 1–10.

258. Scharsack JP, Kalbe M, Derner R, Kurtz J, Milinski M (2004) Modulation of granulocyte responses in three-spined sticklebacks Gasterosteus aculeatus infected with the tapeworm Schistocephalus solidus. *Diseases of aquatic organisms*, **59**, 141–50.

259. Scharsack J, Kalbe M, Harrod C, Rauch G (2007) Habitat-specific adaptation of immune responses of stickleback (Gasterosteus aculeatus) lake and river ecotypes. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 1523–1532.

260. Schluter D (1995) Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology*, **76**, 82–90.

261. Schluter D (1996) Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **351**, 807–814.

262. Schluter D (2000) *The ecology of adaptive radiation*. Oxford University Press, Oxford, UK.

263. Schluter D (2001) Ecology and the origin of species. *Trends in Ecology & Evolution*, **16**, 372–380.

264. Schluter D (2009) Evidence for ecological speciation and its alternative. *Science*, **323**, 737–41.

265. Schluter D, Conte GL (2009) Genetics and ecological speciation. *Proceedings of the National Academy of Sciences of the United States of America*, **106 Suppl** , 9955–62.

266. Schmid-Hempel P, Ebert D (2003) On the evolutionary ecology of specific immune defence. *Trends in Ecology & Evolution*, **18**, 27–32.

267. Schulenburg H, Kurtz J, Moret Y, Siva-Jothy MT (2009) Introduction. ecological immunology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 3–14.

268. Schulte RD, Makus C, Hasert B, Michiels NK, Schulenburg H (2010) Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 7359–64.

269. Schulte RD, Makus C, Schulenburg H (2013) Host–parasite coevolution favours parasite genetic diversity and horizontal gene transfer. *Journal of Evolutionary Biology*, **26**, 1836–1840.

270. Servedio M (2001) Beyond reinforcement: the evolution of premating isolation by direct selection on preferences and postmating, prezygotic incompatibilities. *Evolution*, **55**, 1909–1920.

271. Servedio MR, Doorn GS Van, Kopp M, Frame AM, Nosil P (2011) Magic traits in speciation: "magic" but not rare? *Trends in ecology & evolution*, **26**, 389–396.

272. Servedio M, Kopp M (2012) Sexual selection and magic traits in speciation with gene flow. *Curr. Zool*, **58**, 510–516.

273. Skinner M, Manikkam M, Haque M (2012) Epigenetic transgenerational inheritance of somatic transcriptomes and epigenetic control regions. *Genome Biology*, **13**, 1–21.

274. Slade RW (1992) Limited MHC polymorphism in the southern elephant seal: implications for MHC evolution and marine mammal population biology. *Proceedings. Biological sciences / The Royal Society*, **249**, 163–71.

275. Smadja CM, Butlin RK (2011) A framework for comparing processes of speciation in the presence of gene flow. *Molecular ecology*, **20**, 5123–40.

276. Sobel J, Chen G (2014) Unification of methods for estimating the strength of reproductive isolation. *Evolution*, **68**, 1–40.

277. Sommer S (2005) The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Frontiers in zoology*, **2**, 1–18.

278. Sommerfeld R, Boehm T, Milinski M (2008) Desynchronising male and female reproductive seasonality: dynamics of male MHC-independent olfactory attractiveness in sticklebacks. *Ethology Ecology & Evolution*, **20**, 325–336.

279. Sorci G (2013) Immunity, resistance and tolerance in bird parasite interactions. *Parasite immunology*, **35**, 350–361.

280. Sorci G, Faivre B (2009) Inflammation and oxidative stress in vertebrate host-parasite systems. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 71–83.

281. Sorci G, Møller AP, Boulinier T (1997) Genetics of host-parasite interactions. *Trends in Ecology & Evolution*, **12**, 196–200.

282. Stelkens RB, Young KA, Seehausen O (2010) The accumulation of reproductive incompatibilities in African cichlid fish. *Evolution*, **64**, 617–33.

283. Stockley P, Gage MJG, Parker GA, Møller AP (1997) Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. *The American Naturalist*, **149**, 933–954.

284. Summers K, McKeon S, Sellars J *et al.* (2003) Parasitic exploitation as an engine of diversity. *Biological reviews of the Cambridge Philosophical Society*, **78**, 639–75.

285. Svensson EI, Råberg L (2010) Resistance and tolerance in animal enemy-victim coevolution. *Trends in ecology & evolution*, **25**, 267–74.

286. Swanson WJ, Vacquier VD (2002) The rapid evolution of reproductive proteins. *Nature Reviews Genetics*, **3**, 137–144.

287. Swarup H (1958) Stages in the development of the stickleback Gasterosteus aculeatus (L.). *Journal of Embryology and Experimental Morphology*, **6**, 373–383.

288. Tennessen JA, Bigham AW, O'Connor TD *et al.* (2012) Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science*, **337**, 64–9.

289. Theis A, Ronco F, Indermaur A, Salzburger W, Egger B (2014) Adaptive divergence between lake and stream populations of an East African cichlid fish. *Molecular ecology*, **23**, 5304–5322.

290. Thompson J (2002) Coevolution and maladaptation. *Integrative and Comparative Biology*, **387**, 381–387.

291. Tidbury HJ, Best A, Boots M (2012) The epidemiological consequences of immune priming. *Proceedings. Biological sciences / The Royal Society*, **279**, 4505–12.

292. Tinbergen N (1952) The Curious Behavior of the Stickleback. *Scientific American*, **187**, 22–26.

293. Tobler M, Plath M, Riesch R *et al.* (2014) Selection from parasites favours immunogenetic diversity but not divergence among locally adapted host populations. *Journal of evolutionary biology*, **27**, 960–74.

294. Tollenaere C, Bryja J, Galan M *et al.* (2008) Multiple parasites mediate balancing selection at two MHC class II genes in the fossorial water vole: insights from multivariate analyses and population genetics. *Journal of evolutionary biology*, **21**, 1307–20.

295. Tschirren B, Andersson M, Scherman K *et al.* (2013) Polymorphisms at the innate immune receptor TLR2 are associated with Borrelia infection in a wild rodent population. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 1–8.

296. Turner LM, Hoekstra HE (2008) Causes and consequences of the evolution of reproductive proteins. *The International journal of developmental biology*, **52**, 769–80.

297. Uller T (2008) Developmental plasticity and the evolution of parental effects. *Trends in Ecology & Evolution*, **23**, 432–438.

298. Uller T, Nakagawa S, English S (2013) Weak evidence for anticipatory parental effects in plants and animals. *Journal of evolutionary biology*, **26**, 2161–70.

299. Vacquier V (1998) Evolution of gamete recognition proteins. *Science*, **1995**.

300. Vale PF (2013) Killing them softly: managing pathogen polymorphism and virulence in spatially variable environments. *Trends in parasitology*, **29**, 417–22.

301. Vale PF, Fenton A, Brown SP (2014) Limiting damage during infection: lessons from infection tolerance for novel therapeutics. *PLoS biology*, **12**, e1001769.

302. Vale PF, Wilson AJ, Best A, Boots M, Little TJ (2011) Epidemiological, evolutionary, and coevolutionary implications of context-dependent parasitism. *The American naturalist*, **177**, 510–21.

303. Van Valen L (1973) A new evolutionary law. *Evolutionary theory*, **30**, 1–30.

304. Van der Ven K, Fimmers R, Engels G, van der Ven H, Krebs D (2000) Evidence for major histocompatibility complex-mediated effects on spermatogenesis in humans. *Human reproduction*, **15**, 189–96.

305. Via S, Bouck A, Skillman S (2000) Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution*, **54**, 1626–1637.

306. Wade MJ, Patterson H, Chang NW, Johnson NA (1994) Postcopulatory, prezygotic isolation in flour beetles. *Heredity*, **72**, 163–167.

307. Wakeland EK, Boehme S, She JX *et al.* (1990) Ancestral polymorphisms of MHC class II genes: divergent allele advantage. *Immunologic research*, **9**, 115–22.

308. Wedekind C, Chapuisat M, Macas E, Rülicke T (1996) Non-random fertilization in mice correlates with the MHC and something else. *Heredity*, **77**, 400–9.

309. Wegner KM (2008) Historical and contemporary selection of teleost MHC genes: did we leave the past behind? *Journal of Fish Biology*, **73**, 2110–2132.

310. Wegner KM, Kalbe M, Kurtz J, Reusch TBHT, Milinski M (2003a) Parasite selection for immunogenetic optimality. *Science*, **301**, 1343.

311. Wegner KM, Reusch TBH, Kalbe M (2003b) Multiple parasites are driving major histocompatibility complex polymorphism in the wild. *Journal of evolutionary biology*, **16**, 224–32.

312. Windsor D (1998) Most of the species on Earth are parasites. *International journal for parasitology*, **28**, 1939–41.

313. Woelfing B, Traulsen A, Milinski M, Boehm T (2009) Does intra-individual major histocompatibility complex diversity keep a golden mean? *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 117–28.

314. Wolf JB, Brodie III ED, Cheverud JM, Moore AJ, Wade MJ (1998) Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution*, **13**, 64–69.

315. Wolf JB, Wade MJ (2009) What are maternal effects (and what are they not)? *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 1107–15.

316. Woolhouse M, Webster J (2002) Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nature genetics*, **32**, 569–577.

317. Wootton RJ (1976) *The biology of the sticklebacks* (New York: Academic Press, Ed,).

318. Wu C-I, Ting C-T (2004) Genes and speciation. *Nature reviews. Genetics*, **5**, 114–22.

319. Yeates SE, Diamond SE, Einum S *et al.* (2013) Cryptic Choice of Conspecific Sperm Controlled By the Impact of Ovarian Fluid on Sperm Swimming Behavior. *Evolution*, **67**, 3523–3536.

320. Yeates SE, Einum S, Fleming I a *et al.* (2009) Atlantic salmon eggs favour sperm in competition that have similar major histocompatibility alleles. *Proceedings. Biological sciences / The Royal Society*, **276**, 559–66.

321. Zahavi A (1975) Mate selection-a selection for a handicap. *Journal of theoretical biology*, **53**, 205–14.

322. Zajitschek S (2014) Short-term variation in sperm competition causes sperm-mediated epigenetic effects on early offspring performance in the zebrafish. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 1–8.

323. Zbinden M, Largiader C, Bakker T (2001) Sperm allocation in the three-spined stickleback. *Journal of Fish Biology*, **59**, 1287–1297.

Appendices

Appendix Chapter I

Electronic supplementary material: Methods

Detailed methods for sperm extraction and analysis

Sperm suspension was prepared by gently mashing both testes through a 40 µm micro-cell strainer sieve (brand) and rinsing the sieve with three times 300 µl Hank's Balanced Salt Solution (Sigma-Aldrich Company, Germany). 300 µl of this solution was collected in a 0.5 mL eppendorf tube. After a 1:2 dilution in well water, 3µl of the resulting suspension was introduced in a counting chamber (standard count 4 chamber slide, 20 µm depth, Leja Nieuw Vennep, Nederlands) under a Olympus CX41 microscope at 100x magnification. To quantify spermatozoa concentration and velocity, we used computer assisted sperm analysis (C.A.S.A.) using a Hamilton-Thorne CEROS camera set-up and the Animal Mobility software (Hamilton Thorne Biosciences, Beverly, MA, USA).We recorded total number of sperm and multiple sperm motion parameters : Curvilinear, straight-line and average-path velocity, beat-cross frequency and lateral head displacement (VCL, VSL, VAP, BCF ALH;(Kime *et al.* 2001)). Sperm characteristics were recorded on five separate areas twice in two slide chambers for each individual (20 measurements per individual, 34-36 per population, n=142). A principal component analysis (PCA) on three sperm motility variables (VSL, VCL, and VAP) was used to obtain an overall measure of sperm velocity. We used the first factor score, which explained 69.5 % of the variance and was strongly correlated with the three velocity parameters (Correlation coefficients: r_{VSL}=0.96, r_{VCL}=0.88, r_{VAP}=0.96). Both sperm concentration and sperm velocity (PC1) correlated significantly with log-transformed testes mass (Pearson's correlation sperm concentration: r (95%CI)=0.746 (0.662-0.811), p<0.0001; sperm velocity: r (95%CI)= 0.199 (0.036-0.353), p=0.017).

Detailed methods for egg genotyping and parentage analysis

For the adult fish, DNA extractions from tail fin were performed using the DNAeasy purification kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA extractions from eggs were performed using the Invisorb® DNA Tissue HTS 96 Kit/ Teckit from Invitek on a Tecan FreedomEvo robot platform. All samples were genotyped for 5 microsatellite loci combined into one multiplex PCR protocol for paternity analysis (Kalbe *et al.* 2009). Allele calls were performed using Genemarker 1.85 (www.softgenetics.com) and parentage analysis using CERVUS v3.0.3 (Field Genetics Ltd; S3 (Kalinowski *et al.* 2007)). The most likely father was estimated based on LOD score ratios between the two putative sires.

Electronic supplementary material S1:

Geographic coordinates of populations of origin

Population name	Habitat	Coordinates
Grosser Plöner lake	Lake	54° 9'21.61"N, 10°25'48.52"E
Malenter Au	River	54°12'15.08"N, 10°33'41.90"E
Westen lake	Lake	54°17'1.92"N, 9°56'55.71"E
Eider river	River	54°15'49.02"N, 10° 2'24.47"E

Electronic supplementary material S2

Detailed table representing the full-factorial experimental design for sperm competition trials within- and between- populations and ecotypes. Each replicate consists of a sympatric mating pair (female + sympatric male) and a competing male. All individuals were used only once to avoid pseudo-replication and sequence effects. (L : Lake, R:River, L1: Grosser Plöner lake, L2: Westen lake, R1: Malenter Au, R2: Eider river).

Female and sympatric male origin	**Competing male origin**	**Competition type**	**Sample size**	**Sample size per population**	**Sample size per ecotype**
L1	L1	Within-ecotype	5	17	32
L1	L2	Within-ecotype	4		
L1	R1	Between-ecotype	4		
L1	R2	Between-ecotype	4		
L2	L1	Within-ecotype	3	15	
L2	L2	Within-ecotype	4		
L2	R1	Between-ecotype	4		
L2	R2	Between-ecotype	4		
R1	L1	Between-ecotype	4	15	32
R1	L2	Between-ecotype	3		
R1	R1	Within-ecotype	4		
R1	R2	Within-ecotype	4		
R2	L1	Between-ecotype	4	17	
R2	L2	Between-ecotype	6		
R2	R1	Within-ecotype	4		
R2	R2	Within-ecotype	3		

Electronic supplementary material S3

Effects of ecotype (lake vs. river) and drainage system on (a) sperm velocity and (b) total sperm concentration. (d.f. : degrees of freedom).

(a) Sperm velocity

	d.f.	F value	p-value
Ecotype	**1,141**	**9.07**	**0.003**
System	1,141	0.26	0.613
Testes mass (log)	1,141	3.88	0.051
Ecotype : System	**1,141**	**4.46**	**0.037**

(b) Sperm concentration

	d.f.	F value	p-value
Ecotype	**1,141**	**9.52**	**0.002**
System	1,141	0.08	0.778
Testes mass (log)	1,141	**162.14**	**<0.001**
Ecotype : System	**1,141**	**9.12**	**0.003**

Electronic supplementary material S4

We conducted retrospective power analysis so that we could evaluate whether the number of replicates was adequate to detect biases in paternity if biases existed. Calculating an effect size for a thorough power analysis of the linear model presented in Table 1 was not possible based on the information available in the literature. We thus performed a more straightforward power analysis showing that the number of replicates was sufficiently high to have had detected differences in paternity within and between treatments. To this end, we calculated the effect size for a simple proportion test (i.e. chi-square test for equality of proportions) based on relevant studies. We used five representative vertebrate studies where sperm competition assays were performed between males from different populations or species (Ludlow & Magurran 2006; Mendelson *et al.* 2007; Martín-Coello *et al.* 2009; Immler *et al.* 2011; Yeates *et al.* 2013). For each treatment within those studies (i.e. each cross-type, N=15), we estimated the number of experimental replicates (mean=8.93, sd=5.9), the average sample size of eggs or ova (mean=18.71, sd=6.74) and the advantage of the conspecific male (mean=0.706, sd=0.27). For each treatment where a significant advantage for the 'conspecific' male (i.e. same population or species as the female, n=10) was reported, we estimated the effect size for a proportion test. The null hypothesis of this test is that the paternity of the conspecific male did not deviate from a random expectation of 50/50. Using h=2*asin(sqrt(conspecific advantage))-2* asin(sqrt (0.5)) (Cohen 1988), we estimated a mean effect size of h=0.835. Using this effect size, we simulated the power provided for a series of simulated number of replicates using the pwr.p.test function in R (pwr package). This showed that, assuming a similar effect size (h=0.835), a power (1-β) of 0.99 can be reached if the replicate number is greater than 25. Our replicate number of 28 and 29 sperm competition trials ought therefore to be sufficient to detect differences from a random expectation of 50/50 at α=0.05 with a probability of power=0.994 and power =0.993, respectively. Furthermore, these experiments have reported significant differences to random expectation between-population or between-species sperm competition trials with smaller replicate number and sample sizes of eggs. We found that no significant deviation from equally shared paternity in both within-ecotype and between-ecotype trials (respectively, χ^2=0.037, df=1, p=0.848 and χ^2=1.049, df=1, p=0.306). The mean proportion of eggs fertilized by the sympatric male did not differ significantly between within-ecotype and between-ecotype trials (χ^2=1.743, df = 1, p-value = 0.187, h=0.037). Overall, this analysis shows that our sample size provided

sufficient power to detect a conspecific sperm precedence effect as large as those reported in the literature.

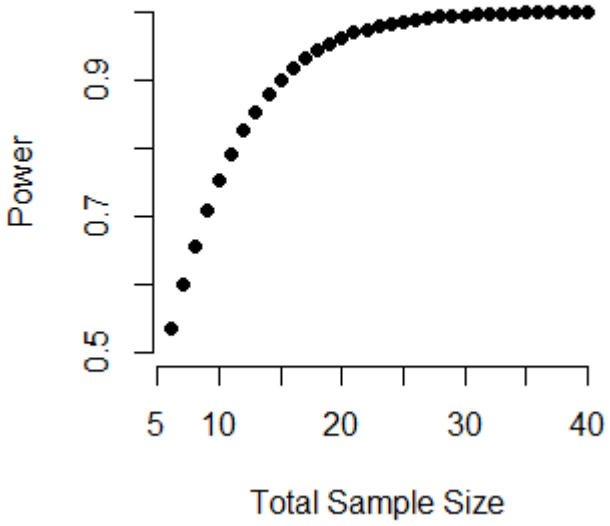


Figure showing the power of a proportion test assuming an effect size of h=0.835 according to simulated total of sample sizes ranging from 6 to 40.

Appendix Chapter II

Table S1 :

Multiple pairwise comparisons (Tukey HSD post-hocs tests) of relative mass change and immune related traits between experimental groups. Shown are the estimate differences and their standard error, each pairwise t-test and the associated degrees of freedom and p-values.

	Contrast	est.	SE	df	t.ratio	p.value
Relative mass change	**Lake resident-River resident**	-18.128	2.345	20.14	-7.730	<.0001
	Lake resident-Lake migrant	-9.841	2.335	19.72	-4.215	0.002
	Lake resident-River migrant	-17.983	4.025	121.12	-4.468	<.0001
	River resident-Lake migrant	8.287	1.513	118.65	5.479	<.0001
	River resident-River migrant	0.145	3.942	65.17	0.037	1.000
	Lake migrant-River migrant	-8.142	3.952	65.46	-2.060	0.177
Splenosomatic index	Lake resident-River resident	0.169	0.079	40.10	2.126	0.162
	Lake resident-Lake migrant	-0.159	0.079	39.08	-2.012	0.201
	Lake resident-River migrant	-0.052	0.155	119.85	-0.331	0.987
	River resident-Lake migrant	-0.327	0.061	120.37	-5.379	<.0001
	River resident-River migrant	-0.220	0.146	92.70	-1.513	0.434
	Lake migrant-River migrant	0.107	0.146	92.91	0.734	0.883
Granulocyte / Lymphocyte ratio	Lake resident-River resident	0.115	0.053	43.66	2.163	0.150
	Lake resident-Lake migrant	0.092	0.052	41.97	1.770	0.302
	Lake resident-River migrant	0.276	0.098	117.32	2.811	0.029
	River resident-Lake migrant	-0.023	0.038	119.69	-0.604	0.931
	River resident-River migrant	0.160	0.092	95.02	1.740	0.309
	Lake migrant-River migrant	0.184	0.092	94.45	1.992	0.198

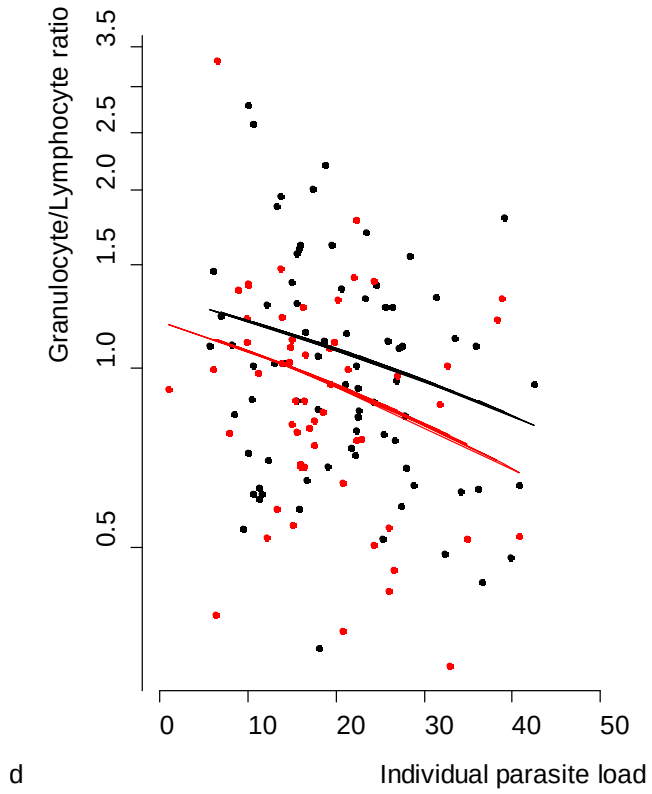


Figure S1:

Effect of individual parasite load on Granulocyte to lymphocyte ratio for fish from lake ecotype (black dots and lines) and river ecotype (red dots and lines).

Table S2:

(a) Parasite prevalence (proportion of fish infected) and (b) mean infection intensity (mean number of parasites in infected fish) in sticklebacks from lake and river ecotype transferred in lake or river habitat.

(a) PREVALENCE	Lake residents	Lake migrants	River migrants	River residents
Protozoa				
Trichodina sp.	0.96	0.86	1.00	0.94
Apiosoma sp.	0.25	0.71	0.33	0.90
Monogenea				
Gyrodactylus sp.	0.39	0.31	0.78	0.44
Digenea				
Diplostomum sp.	1.00	0.90	1.00	0.48
Apatemon cobitis	0.75	0.67	0.11	0.06
Cyathocotyle prussica	0.96	0.98	0.78	0.92
Echinochasmus sp.	0.50	0.51	0.44	0.25
Tylodelphis clavata	0.07	0.02	0.22	0.00
Cestoda				
Valipora campylancristrota	0.00	0.02	0.00	0.00
Paradilepis scolecina	0.00	0.04	0.00	0.00
Proteocephalus filicollis	0.36	0.02	0.11	0.02
Nematoda				
Camallanus lacustris	0.79	0.24	0.33	0.00
Anguillicoloides crassus	0.04	0.02	0.11	0.17
Contracaecum sp.	0.36	0.33	0.22	0.00
Raphidascaris acus	0.00	0.08	0.11	0.38
Crustacea				
Argulus foliaceus	0.32	0.00	0.78	0.00
Ergasilus sp.	0.04	0.00	0.00	0.02
Acanthocephala				
Acanthocephalus sp.	0.00	0.04	0.00	0.10
Mollusca				
Glochidia sp.	0.82	0.04	0.67	0.00

(b) INTENSITY	Lake residents	Lake migrants	River migrants	River residents
Protozoa				
Trichodina sp.	NA	NA	NA	NA
Apiosoma sp.	NA	NA	NA	NA
Monogenea				
Gyrodactylus sp.	2.36	2.93	9.43	12.14
Digenea				
Diplostomum sp.	7.54	3.95	16.00	1.43
Apatemon cobitis	4.52	5.67	1.00	2.67
Cyathocotyle prussica	7.33	8.46	3.00	3.00
Echinochasmus sp.	3.43	6.44	5.50	5.00
Tylodelphis clavata	3.00	1.00	1.00	-
Cestoda				
Valipora campylancristrota	-	1.00	-	-
Paradilepis scolecina	-	1.00	-	-
Proteocephalus filicollis	1.20	1.00	1.00	1.00
Nematoda				
Camallanus lacustris	3.91	2.75	1.67	-
Anguillicoloides crassus	1.00	1.00	2.00	1.13
Contracaecum sp.	1.80	1.19	1.00	-
Raphidascaris acus	-	1.00	1.00	1.22
Crustacea				
Argulus foliaceus	1.78	-	3.71	-
Ergasilus sp.	1.00	-	-	1.00
Acanthocephala				
Acanthocephalus sp.	-	1.00	-	1.00
Mollusca				
Glochidia sp.	4.96	1.50	6.17	-

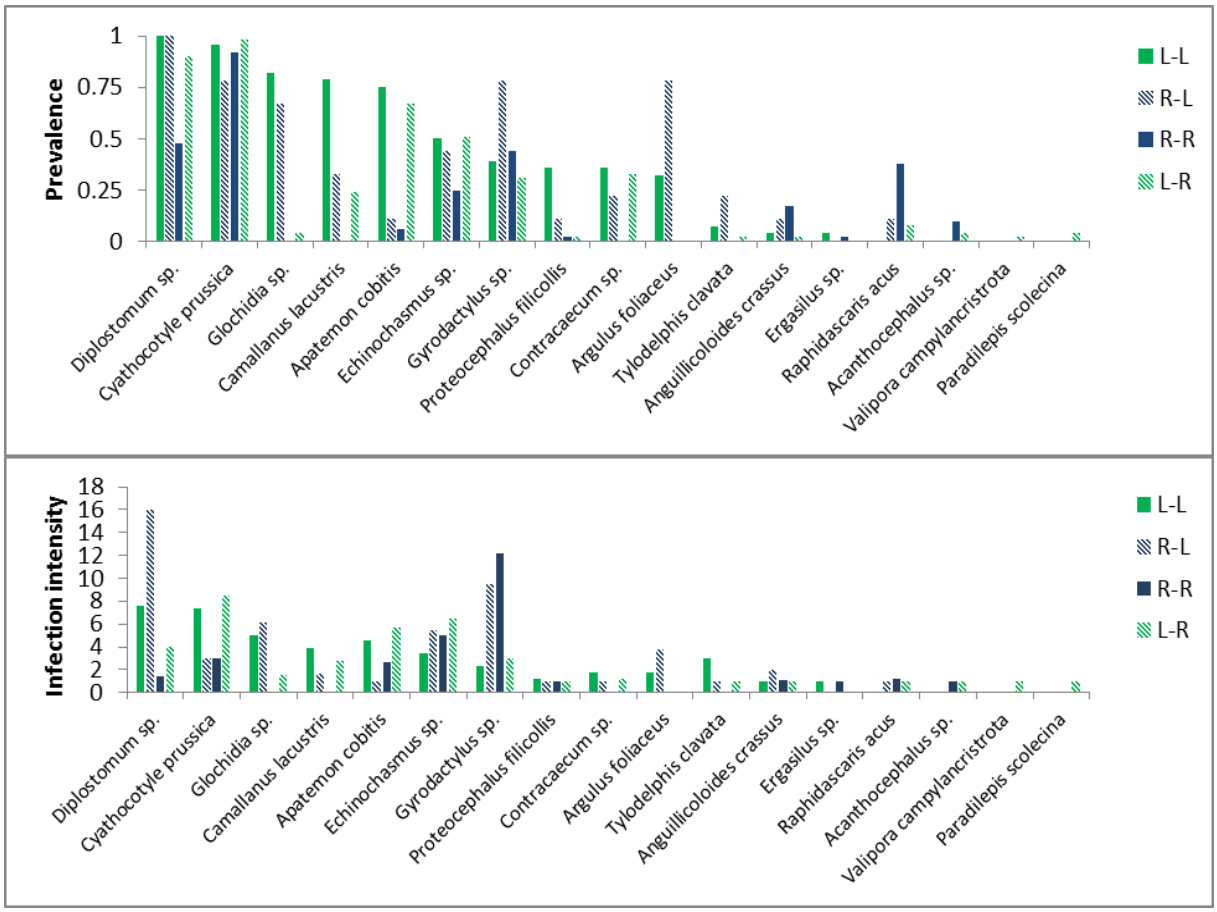


Fig. S2 :

Differences in parasite prevalence (proportion of fish infected) and mean infection intensity (mean number of parasites in infected fish) in sticklebacks from lake and river ecotype transferred in lake or river habitat.

Table S3:

Analyses of variance tables of models on the effects of MHC diversity (a:intraindividual allelic divergence, b:individual allele number), habitat of exposure and migration treatment on parasite diversity and individual parasite load. F-statistic and denominator df values associated were corrected with the Kenward-Roger approximation.

(a)	Shannon Index		Parasite load	
	F	Pr(>F)	F	Pr(>F)
MHC p-distance	2.260	0.136	0.053	0.818
Habitat	**40.842**	**<0.001**	**12.732**	**0.006**
Migration	1.593	0.209	0.008	0.931
Habitat: p-distance	0.007	0.932	0.321	0.572
Migration:p-distance	1.036	0.311	0.436	0.511
Habitat: Migration	**5.198**	**0.025**	0.009	0.921
Migration: Habitat:p-distance	0.004	0.948	0.492	0.484

(b)	Shannon Index		Parasite load	
	F	Pr(>F)	F	Pr(>F)
MHC allele number	0.442	0.778	0.743	0.565
Habitat	**28.628**	**0.0004**	1.663	0.200
Migration	0.682	0.411	2.212	0.141
Habitat: allele number	1.107	0.349	0.095	0.963
Migration: allele number	1.139	0.337	1.539	0.209
Habitat: Migration	**9.869**	**0.002**	**12.149**	**0.006**
Migration: Habitat: allele number	0.664	0.517	1.205	0.304

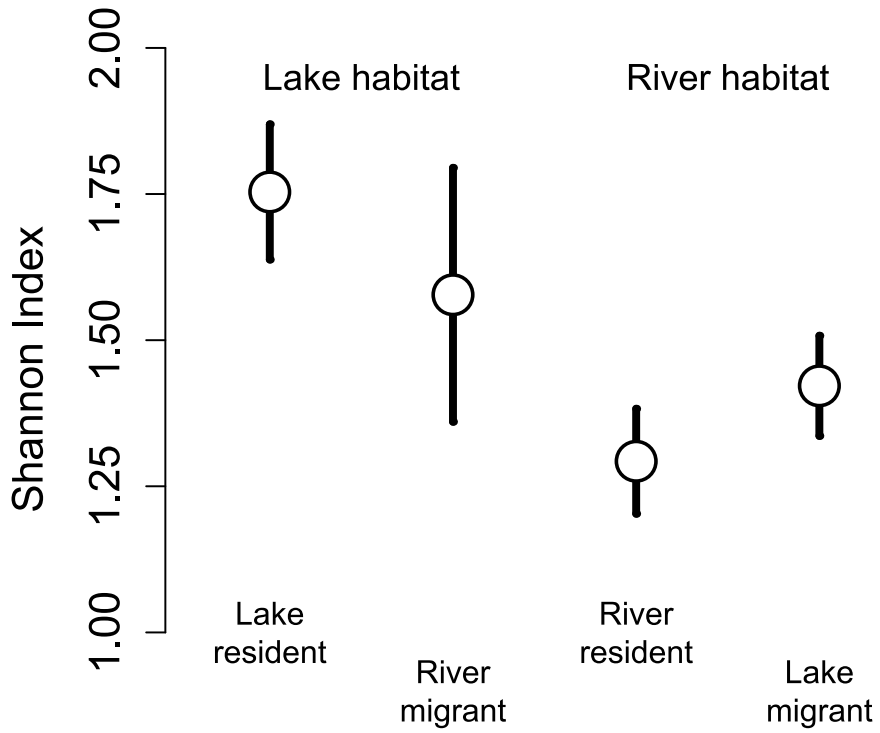


Fig. S3 :

Differences in parasite diversity (Shannon's diversity index) (means +-0.95 CI) in sticklebacks from lake and river ecotype transferred in lake or river habitat

Table S4:

Results from the multivariate permutational analysis (PERMANOVA) of differences in log transformed parasite abundance between treatments.

	Df	F	Pr(>F)
Habitat	1	34.501	0.001
Migration	1	15.895	0.001
Habitat:Migration	1	16.075	0.001
residuals	122		

Table S5 :

Results from the multivariate permutational analysis (PERMANOVA) of differences in MHC IIB haplotype pools between live and dead migrants and residents from lake and river ecotypes.

Lake ecotype	Df	SS	F	R^2	P(>F)
Migration	**1.00**	**8.98**	**32.45**	**0.26**	**0.001**
Survival	1.00	0.28	1.01	0.01	0.42
Migration:Survival	1.00	0.16	0.58	0.004	0.71
residuals	89.00	24.63		0.72	
Total	92.00	34.05			

River ecotype	Df	SS	F	R^2	P(>F)
Migration	**1.00**	**9.83**	**37.55**	**0.29**	**0.01**
Survival	1.00	0.39	1.01	0.01	0.17
Migration:Survival	1.00	0.37	0.58	0.01	0.27
residuals	90.00	23.55		0.72	
total	93.00	34.14			

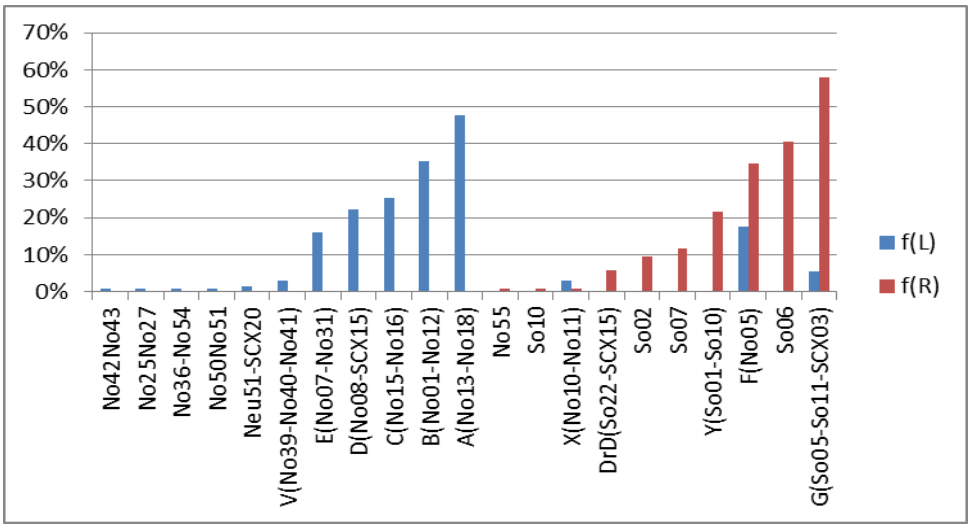


Figure S4 :

MHC class IIB haplotype frequency estimates in lake and river sticklebacks. We detected 21 haplotypes in total (11 lake-specific, 7 river-specific and 3 shared by both fish ecotypes). The shared haplotypes displayed obvious ecotype differences in frequencies. MHC IIB differed significantly between ecotypes in term of allele numbers, allelic divergence and haplotype pools (ANOSIM, R=0.417, p=0.001), with significantly higher allelic divergence and allele numbers in lake fish compared to river fish (respectively, t=2.55, p=0.012 and χ^2=4.091, p=0.043).

Table S6:

Differences in allelic diversity (MHC p-distance and allele number) between experimental groups within ecotype. Differences were tested with generalized linear models based on a Poisson distribution (log function) with type II Chi-square based likelihood-ratio tests.

	Lake fish		River fish	
	MHC p-distance		MHC p-distance	
	F	p	F	p
Migration (resident/migrant)	0.626	0.431	0.774	0.381
Survival (yes/no)	1.158	0.285	0.299	0.586
Migration : Survival	1.146	0.287	0.131	0.719
	Allele number		Allele number	
	χ^2	p	χ^2	p
Migration (resident/migrant)	0.559	0.455	0.287	0.592
Survival (yes/no)	0.032	0.858	0.053	0.817
Migration : Survival	0.158	0.691	0.596	0.440

Table S7 :

Type II analyses of variance tables of linear generalized models on the effects of MHC haplotype and habitat of exposure on infection intensity of three major parasites (*Cyathocotyle prussica*, *Diplostomum sp.* and *Gyrodactylus sp.*). Chi-square values based on likelihood-ratio tests.

	Haplotype A			**Haplotype F**			**Haplotype G**		
		χ^2	p		χ^2	p		χ^2	p
Cyathocotyle prussica	Haplotype A	48.27	<0.001	Haplotype F	7.43	0.01	Haplotype G	1.27	0.26
	Habitat	6.65	0.01	Habitat	3.98	0.05	Habitat	2.79	0.10
	Haplotype A:Habitat	59.90	<0.001	Haplotype F:Habitat	12.03	<0.001	Haplotype G:Habitat	2.61	0.11
		χ^2	p		χ^2	p		χ^2	p
Diplostomum sp.	Haplotype A	2.45	0.12	Haplotype F	1.64	0.20	Haplotype G	3.27	0.07
	Habitat	157.29	<0.001	Habitat	159.41	<0.001	Habitat	159.21	<0.001
	Haplotype A:Habitat	35.38	<0.001	Haplotype F:Habitat	2.54	0.11	Haplotype G:Habitat	26.99	0.00
		χ^2	p		χ^2	p		χ^2	p
Gyrodactylus sp.	Haplotype A	85.20	<0.001	Haplotype F	3.23	0.07	Haplotype G	51.24	<0.001
	Habitat	15.69	<0.001	Habitat	12.28	<0.005	Habitat	9.96	<0.005
	Haplotype A:Habitat	3.78	0.05	Haplotype F:Habitat	6.72	0.01	Haplotype G:Habitat	0.34	0.56

Table S8:

Pairwise post-hocs tests for generalized models on the effects of MHC haplotype and habitat of exposure on infection intensity of two major parasites (*Cyathocotyle prussica* and *Diplostomum sp.*). Odds ratios tests as post-hoc tests. R signifies a relation of resistance between parasite intensity and haplotype and S susceptibility.

	Cyathocotyle prussica and A				*Cyathocotyle prussica and F*		
	z ratio	p			z ratio	p	
Lake	2.867	0.022	R		4.131	0.0002	R
River	-5.233	<.0001	S		-1.919	0.22	S
	Diplostomum sp. and A				*Diplostomum sp. and G*		
	z ratio	p			z ratio	p	
Lake	3.19	0.008	R		-4.686	<.0001	S
River	-9.851	<.0001	S		2.946	0.017	R

Table S9:

Correlation matrix between the abundance of two major parasites (*Cyathocotyle prussica*, *Diplostomum sp.*) and condition-related traits. Shown are Spearman's ρ and the p-value associated with the spearman correlation test.

		H.S.I.	Growth	Condition
Cyathocotyle	Rho	-0.198	-0.266	-0.072
		p=0.027	p=0.003	p=0.42
Diplostomum	Rho	-0.324	-0.287	-0.205
		p<0.001	p=0.003	p=0.037

Table S10: List of MHC II B haplotypes found in this study, including alleles composing the haplotype and known accessions numbers.in GenBank

Haplotype name	Allele 1	accession number 1	Allele 2	accession number 2	Allele 3	accession number 3
A	No13	AF395711	No18	AY687846		
B	No01	DQ016399	No12	DQ016400		
C	No15	DQ016410	No16	DQ016417		
D	No08	AY687842	SCX15	EU541449		
E	No07	AF395718	No31	GQ277654		
F	No05	AY687829				
G	So05	DQ016402	So11	DQ016404	SCX03	AJ230191
H.DrD	S022	NA	SCX15	EU541449		
H.V	No39	NA	No40	NA	No41	NA
H.X	No10	AF395722	No11	AY687843		
H.Y	So01	FJ360535*	So10	FJ360534*		
Neu51.SCX20	Neu51	AY687833	SCX20	FJ360541*		
No25No27	No25	NA	No27	NA		
No36.No54	No36	NA	No54	NA		
No42No43	No42	FJ360536*	No43	FJ360532*		
No50No51	No50	NA	No51	NA		
No55	No55	NA				
So02	So02	DQ016426				
So06	So06	FJ360531*				
So07	So07	NA				
So10	So10	FJ360534*				
No45	No45	FJ360537*				
No46	No46	FJ360538*				
No48	No48	FJ360539*				
No49	No49	FJ360540*				

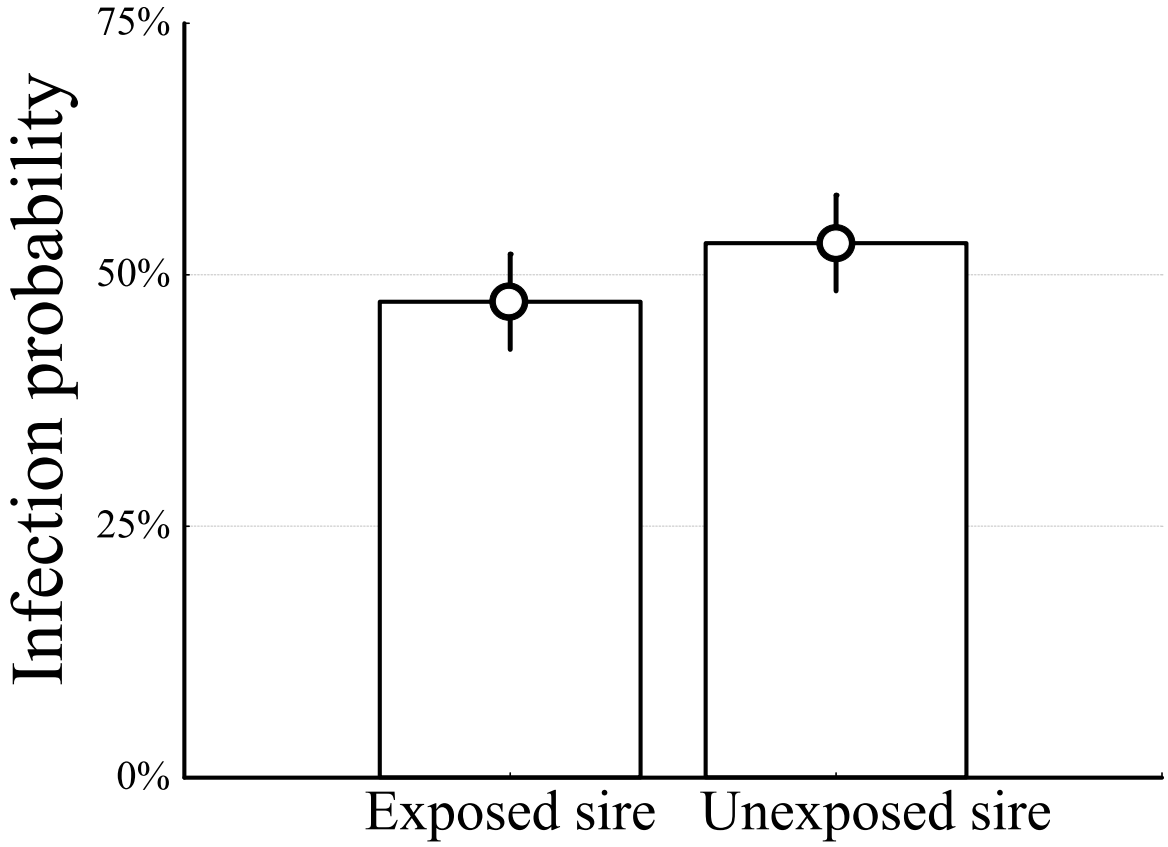
Appendix Chapter III

Supplementary figure S1:

Stickleback eggs at day 5 post fertilization: fully developed embryos, one dead undeveloped zygote and an unfertilized oocyte.

Supplementary figure S2:

Trans-generational effects of paternal parasite exposure on infection probability in G2 fish generation (mean probability of being infected when exposed). Shown are means of infection probability (no significant differences) in G2 offspring sired by either exposed or unexposed fathers. Error bars represent ±1 SE.



Supplementary figure S3:

Trans-generational effects of paternal parasite exposure on body condition at the end of the experiment. Bars are means (±1 SE) of body condition (residuals from the regression of body mass on body length) for offspring sired by either exposed or unexposed fathers. As offspring body condition is normalized around zero, the boxes represent the differences from the mean body condition in all offspring.

Supplementary table S1: Differences in male mortality or reproductive behavior.

Because parasite exposure is known to affect mortality and reproductive behaviour, our parasite exposure treatment in the G1 generation may be associated with unintended selection bias in male quality between the treatment groups. In order to test for this unintended bias, we tested for differences between exposed and unexposed G1 males in mortality, nest building behaviours, or the manifestation of courtship behaviour. Parasite exposure did not significantly affect the time needed to build a high quality nest (exposed males: 15.6 days ± SD 1.02, unexposed males: 15.2 days ± SD 1.10, $t_{1,121}$=0.263, p=0.79). Given an effect size of d=1.372 (derived from Rushbrook & Barber (2006)), our sample size of 121 individuals ought to be sufficient to detect differences in nesting behaviour at α=0.05 with a probability of β=0.999 if it had existed. Furthermore, comparable experiments have reported significant effects on nest building behaviour with smaller sample sizes (Rushbrook & Barber 2006; Jäger *et al.* 2007; Macnab *et al.* 2009). Overall, 8 of the 71 exposed G1 males and 6 of the 62 unexposed G1 males were excluded or died over the course of the experiment (χ^2=0.09, p=0.77), suggesting very limited (if any) bias in overall paternal quality resulting from the parasite treatment. Shown in table are the relative frequencies, the odds ratio for each category and the p-value associated with a Fisher exact test. Numbers in brackets represent the number of males that were not used in the experiment over the number of males affected in each treatment.

	Exposed males	**Unexposed males**	**Odds ratio**	**p-value**
No nest building	2.9 % (2/67)	1.8 % (1/57)	1.723	1
No courtship behaviour	3.1 % (2/65)	0 % (0/56)	Inf.	0.499
Died	5.6 % (4/71)	8.1 % (5/62)	0.681	0.733

Supplementary table S2: Test for over-dispersion in Generalized Linear Mixed Models

Because of the intrinsic distribution of parasites in a population, we tested for over-dispersion (excess of residual variance) in our two models including non-normal data (binomial and count) and mixed effects (maternal half-sibship effect).–We did not find a significant difference between the models reported and the same models with an additional observation-level random effect (Infection probability: χ_1=0, p=0.999, Infection intensity: χ_1=0.001, p=0.975). In addition, we report in the following table the residual degrees of freedom (rdf), the sum of squared Pearson residuals (SS residuals), the ratio of SS residuals to rdf and the p-value based on the χ^2 distribution. For each model, the ratio was close to 1 and no significant excess of variance was found.

	rdf	SS residuals	Ratio	p-value
Infection rate model	217	199.11	0.918	0.803
Infection intensity model	107	97.454	0.911	0.735

Supplementary table S3:

Correlation between life history traits: cost of infection in exposed individuals, juvenile mortality, zygote mortality, motile spermatozoa [spz] concentration. Shown are Spearman Rho estimate (below the diagonal) and the associated p-value (above the diagonal). Motile sperm concentration was estimated at the father level and early life history traits were estimated at the family level (n=96-100 families). The cost of infection was calculated as the mean difference in body condition between infected and uninfected individuals per family (n=27 families).

	Infection cost	Juvenile mortality	Zygote mortality	Motile spz concentration
Infection cost		0.311	0.463	0.520
Juvenile mortality	-0.24		0.084	0.128
Zygote mortality	-0.15	0.17		0.511
Motile spz concentration	-0.13	0.13	0.07	

Supplementary table S4: Effects of paternal exposure on the likelihood of being infected.

Statistical table showing results of the likelihood ratio test between two generalized linear models with or without paternal infection as fixed factor. (d.f. : degrees of freedom, AIC : Akaike information criterion, BIC: Bayesian information criterion)

	d.f.	AIC	BIC	logLik	$\chi 2$	P(>χ)
Without paternal infection	4	303.1	316.8	-147.6		
With paternal infection	6	303.5	323.9	-145.8	3.599	0.165

Variation due to maternal half sibship identity : 42.92%	
n=223	groups=15

Supplementary table S5: Effects of paternal exposure on parasite load.

Statistical table showing results of the likelihood ratio test between two generalized linear models with or without paternal infection as fixed factor. (d.f. : degrees of freedom, AIC : Akaike information criterion, BIC: Bayesian information criterion)

	d.f.	AIC	BIC	logLik	$\chi 2$	P(>χ)
Without paternal infection	4	110.3	121.21	-51.15		
With paternal infection	6	114.2	130.6	-51.12	0.061	0.970

Variation due to maternal half sibship identity : 19.07%	
n=113	groups=15

Supplementary table S6:

Effects of paternal exposure, offspring exposure (exposed vs. control) and sex on individual body condition. The table shows the outcome of a linear mixed effect model on individual body condition at the end of the experiment. The variation attributed to the random effect was estimated based on the ratio of the variance due to this effect over the total variance (d.f.: degrees of freedom).

Effect	d.f.	F value	P
Paternal exposure	**1, 471**	**8.737**	**0.003**
Offspring exposure	**1, 471**	**6.418**	**0.012**
Offspring sex	1, 471	0.267	0.606
Paternal exposure x Offspring exposure	1, 471	0.342	0.559
Maternal half sibship (random effect)	38.41%		

Supplementary table S7: Effect of various selection strengths on statistical evidence for paternal effects.

Linear mixed effect models on offspring body condition included the paternal exposure, offspring infection (exposed-infected vs. exposed-non-infected), sex and the interaction between paternal exposure and offspring infection. We simulated selection by excluding a percentage of G2 offspring sired by unexposed fathers to evaluate the strength of selection associated with early life mortality in offspring sired by exposed fathers. This percentage (i.e. selection levels) varied from 5 to 34.8 %, the latter representing twice the relative difference in overall survival between offspring from exposed and unexposed fathers (17.4%). Accordingly, each model was based on a subset of data after *in silico* mortality simulation.

In (a), we removed a proportion of the most infected offspring (based on infection rate) of unexposed fathers, conservatively postulating that selection could have removed the most susceptible offspring sired by exposed fathers. P-value estimates were based on the result of all possible subsets, as individuals with equal infection rates were also randomly excluded. In (b) and (c), we randomly excluded between 5 and 34.8% of either exposed (b) or infected (c) offspring sired by unexposed fathers. Mean p-values and 95% confidence intervals were calculated for each selection level and each scenario, based on 999 models for randomly produced subsets. We report p-values for the main paternal effect and for condition-dependence (interaction effect). Significant effects are highlighted in bold.

Results show that the main paternal effect was statistically robust, even at high levels of selection in every case scenario. The interaction effect was supported by statistical trends even at high levels of selection (up to 20%), showing a complex interplay between selection and paternal effects on the expression of parasite tolerance.

Selection level (%)	(a) Selection based on high infection rate		(b) Random selection amongst exposed		(c) Random selection amongst infected	
	Main effect	Interaction	Main effect	Interaction	Main effect	Interaction
0	**0.005**	**0.043**	n/a	n/a	n/a	n/a
5	**0.007**	0.055	**0.007 ± 0**	0.054 ± 0.001	**0.006 ± 0**	**0.046 ± 0.001**
10	**0.010 ± 0.001**	0.068 ± 0.011	**0.009 ± 0**	0.062 ± 0.002	**0.007 ± 0**	**0.049 ± 0.001**
15	**0.008 ± 0.001**	0.061 ± 0.005	**0.012 ± 0.001**	0.073 ± 0.003	**0.009 ± 0**	0.052 ± 0.001
17.4*	**0.010 ± 0**	0.071 ± 0.003	**0.014 ± 0.001**	0.080 ± 0.004	**0.012 ± 0**	0.059 ± 0.002
20	**0.012 ± 0**	0.081 ± 0.002	**0.014 ± 0.001**	0.082 ± 0.004	**0.014 ± 0.001**	0.063 ± 0.002
34.8§	**0.047 ± 0.004**	0.187 ± 0.014	**0.030 ± 0.003**	0.126 ± 0.008	**0.023 ± 0.001**	0.078 ± 0.003

*: Equivalent selection ; §: Double selection

Declaration

Hereby I declare that

1) Apart from my supervisor's guidance, the content and design of this dissertation is the product of my own work. The co-author's contributions to specific paragraphs are listed in the thesis outline section

2) This thesis has not been submitted either partially or wholly as part of a doctoral degree to another examination body, and no other materials are published or submitted for publication than indicated in the thesis

3) the preparation of the thesis has been subjected to the Rules of Good Scientific Practice of the German Research Foundation.

Kiel, 13th of January 2015

Joshka Kaufmann