# Concomitant natural and sexual selection reveals context-dependent evolution of host resistance to parasites

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To my Grandfather

Dr. Günter Lingenberg

**Abstract**

Where parasites evolve to exploit, hosts evolve to resist. In nature, such coevolutionary dynamics play out in an environment with multiple concurrent selection pressures. This thesis describes the evolutionary benefit of evolved adaptive resistance to parasites and explores experimentally whether resistance can be maintained in spite of competing selective agents. Using three-spined stickleback as a model organism, I, together with colleagues, first demonstrate that under strong parasite-mediated selection the evolution of acquired immunity provides a significant reproductive advantage (Chapter 1). Next, I evaluate how predation impacts host-parasite dynamics, with a special focus on host resistance associated with polymorphism of genes of the major histocompatibility complex (MHC). I show that predation weakens parasite-mediated selection and in the process disrupts negative-frequency dependent selection on MHC haplotypes across generations (Chapter 2). Furthermore, predation can interfere with MHC-based mate choice behaviour (Chapter 3). Expanding on the evidence of context-dependent resistance to parasites, I describe how the link between parasites and specific MHC haplotypes differs seasonally and in accordance with the strength of parasite–mediated selection (Chapter 4). In the last chapter, I experimentally assemble populations with and without MHC haplotypes associated with resistance and observe whether sexual selection differs between the populations. As expected, individuals with resistance-assocaited MHC haplotypes experienced reduced burden of a specific parasite and, as consequence, increased individual lifetime reproductive success (Chapter 5). Collectively, these results underscore the importance of evolved parasite resistance for individual fitness in general. Our findings show just how context-dependent the evolution of resistance can be, with seasonal variation, concomitant predation or different strengths of sexual selection, all affecting the outcome of parasite-mediated selection and selection on immune genes. This work sheds light on why variation in the capacity to resist parasite infection exists among populations within a species or between species.

**Acknowledgments**

Primarily, I would like to thank my primary supervisor, Christophe Eizaguirre. I am particularly grateful for his patience, trust and professional guidance throughout the years and across many collaborative projects. I feel privileged having had the chance to re-live part of his PhD experience through the collaborating with Prof. Manfred Milinski at the Max-Planck Institute for evolutionary biology in Plön, Germany. At this point I would also like to thank Manfred for his contribution. I also want to especially thank Martin Kalbe – a mentor remembered for instilling enthusiasm and a genuine love of science in his students.

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Lastly, I want to express the sincerest gratitude to my partner Calum Melville for his love, patience and endless support. Our banter and laughs gave me strength and your empathy motivated me to push on. You make me very happy.

*Patience is bitter, but its fruit is sweet*.

**— Aristotle**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AIC | Akaike information criterion |
| ANOSIM | Analysis of Similarity |
| $I_{PI}$ | Individual parasite load |
| HLA | human leukocyte antigen |
| LRS | Lifetime reproductive success |
| MHC | Major Histocompatibility complex |
| NCBI | National Centre for Biotechnology Information |
| NFDS | Negative frequency-dependent selection |
| PERMANOVA | Permutational multivariate analysis of variance |
| pop | Population |
| QMUL | Queen Mary University of London |
| RSCA | Reference Strand-mediated Conformation Analysis |
| SE | Standard error |
| SIMPER | Analysis of Similarity Percentage |

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## DETAILS OF COLLABORATION

*Chapter 1:* Christophe Eizaguirre (CE) helped with revisions of this chapter. I (DWS) wrote the first draft and implemented final changes.

*Chapter 2:* Martin Kalbe (MK), Manfred Milinski (MM), and CE conceived and performed the experiment. MK and CE collected the data. MM provided laboratory equipment and material. DWS performed the parenthood analyses and analyzed the data with CE and MK. DWS and CE drafted the manuscript. All authors contributed to the final version of the manuscript.

*Chapter 3/4:* MM conceived the study. MK, CE, MM and Tobias Lenz designed the experiment. MK and CE performed the experiment. MM provided laboratory equipment and material. DWS performed the parenthood analysis and analyzed the data with CE. DWS and CE drafted the manuscript. All authors contributed to the final version of the manuscript.

*Chapter 5:* DWS, MK and CE conceived the experiment. DWS and MK reared fish and set up the transplant experiment. DWS, MK, Marc Ritter and Noemie Erin collaborated on dissections. DWS performed all molecular work. MM provided laboratory equipment and material. DWS and CE analyzed and wrote the manuscript. All authors contributed to the final version of the manuscript.

*Chapter 6:* DWS and CE conceived the study. DWS and CE designed the experiment. DWS and MK performed the experiment and collaborated on dissections. DWS performed all molecular work. MM provided laboratory equipment and material. DWS and CE analyzed the data. DWS and CE wrote the paper. All authors contributed to the final version of the manuscript.

*Chapter 7:* CE suggested revisions of this chapter. DWS wrote the first draft and implemented final changes.

## PUBLICATIONS AND PRESENTATIONS ARISING FROM THESIS CHAPTERS

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3. Schmid DW, Ritter M, Erin N, Milinski M, Kalbe M, Eizaguirre C (*in prep*). MHC-based resistance changes as parasite-mediated selection varies temporally.
4. Schmid DW, Milinski M, Kalbe M, Eizaguirre C (*in prep*). Variation at immuno-genes predictably changes female mate choice.

Oral

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2. Stickleback Sex – Finding Romance in a World of Predators and Parasites. *London Freshwater Group.* The Linnaean Society London, March 2017.
3. Assessing the Darwinian costs of mounting an adaptive immune response. *The British Society for Parasitology*. University of Aberystwyth, April 2018.
4. *Welcome address*: Talking Evolution – on principle and advances in evolutionary thinking. *Talking Evolution.* Max Planck Institute for Evolutionary Biology, September 2018.

Poster

1. Sex under pressure – Predation affects the outcome of parasite-mediated sexual selection for optimal immune genes. *The International Society for Behavioral Ecology*. University of Exeter, August 2016.
2. Predation modifies parasite-mediated mate choice for immune genes. *The European Society for Evolutionary Biology*. University of Groningen, August, 2017.

Costs and benefits of adaptive immunity depend on the strength of parasite-mediated selection. *Evolution*. Montpellier, 2018.

# CHAPTER 1. GENERAL INTRODUCTION

## Statement of Intent

This doctoral project builds on previous work exploring parasite-mediated natural and sexual selection on genes of the Major Histocompatibility complex (MHC). Using various experiments my coworkers and I explored coevolutionary dynamics in complex environmental settings and aimed to understand drivers and constraints to the maintenance of MHC polymorphism.

## Abstract

In this chapter I describe one of the major mechanisms maintaining biodiversity – coevolution. Focusing especially, but not exclusively, on coevolutionary dynamics between host and parasites, I describe two major mechanisms that mediate reciprocal adaptation: negative frequency-dependent selection (NFDS) and arms race dynamics. I expose how sexual selection and mate choice in particular contribute to the evolution of host resistance and coevolutionary dynamics. Literature research demonstrated a strong focus on experimental work studying resistance evolution in simplified systems under laboratory conditions and revealed a knowledge gap about coevolution in complex environments under concomitant selection pressures. Thereafter, I discuss how to address this gap by focusing on the best known genetic basis for parasite resistance and mate choice alike: the genes of the major histocompatibility complex (MHC). I finish by describing natural and sexual selection for individual MHC diversity and specific MHC alleles and why the three-spined stickleback (*Gasterosteus aculeatus*) is a particularly well-suited organism to address our research objectives. Lastly, I summarize how the different chapters presented in this thesis will fill gaps in our understanding of coevolution under concomitant selection pressures.


**Figure 1. A) Hummingbirds (Trochilidae) coevolve with ornithophilous flowers (Kay et al. 2005). B) Coral-zooxanthellae symbiosis arises from coevolution. C) Heliconiine butterfly larvae circumvent morphological and chemical defenses of *Passiflora* plants (De Castro et al. 2018). D) Cestodes such as *Schistocephalus solidus* exploit their hosts (Milinski 1984). Sexually antagonistic selection mediated via the seminal fluid in *Drosophilia melanogaster* alters male fitness relative to female fitness (Chapman et al. 1995).**

**Coevolution and the 'tangled bank'**

Cases of coevolution are intriguing. Coevolution is the process by which two or more organisms evolve in concert and impose reciprocal selection on each other (Thompson 1994, 2013). Traditionally, coevolution was thought of in terms of entomo- or ornithophilous flowering plants (Figure 1A, Darwin 1862; Ehrlich and Raven 1964), but numerous examples as intricate as those of corals and their algae symbionts have been reported since (Figure 1B, Rowan and Knowlton 1995; Brockhurst and Koskella 2013). Such mutualistic coevolutionary relationships, where both coevolving taxa benefit, is one possible outcome of coevolution. Antagonistic coevolution (Figure 1C-E), where one entity exploits the other and where the exploited evolves to counter exploitation, is equally common in nature, with host-parasite relationship being one of the best studied relationships. And yet, understanding coevolution under a complex suite of selection pressures is notoriously difficult and forms a major gap in our knowledge (Wolinska and King 2009; Betts et al. 2016). This thesis aims to explore experimentally how organisms evolve and coevolve as part of a *'[…] tangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, […] elaborately constructed forms so different from each other, and dependent upon each other in so complex a manner […]'* (Darwin 1859).

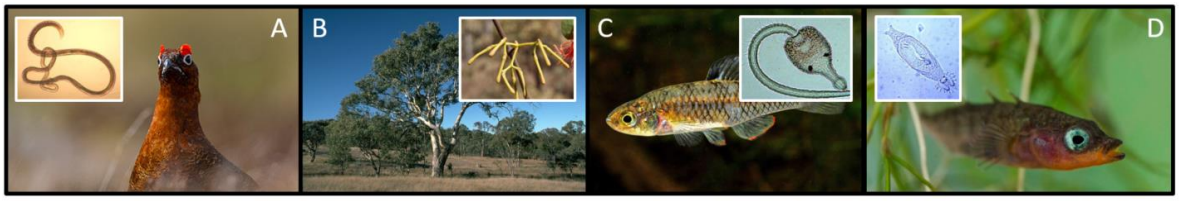


**Figure 2 A) The fecundity of red grouse *Lagopus lagopus scoticus* declines when individuals are infected with the nematode parasite *Trichostringylus tenuis* (Cattadori et al. 2005). B) Nutrient enrichment in the mistletoe *Amyema miguelii* affects element returns to its Eucalyptus tree host (March and Watson 2010). C) Infection with the trematode *Euhaplorchis californiensis* increases predation risk for killifish *Fundulus parvipinnis* (Lafferty & Morris 1996). D) Local differences in prevalence of the ectoparasitic *Gyrodactylus gasterostei* limits migration of three-spined sticklebacks *G. aculeatus* between water sheds (Eizaguirre et al. 2011).**

HOST-PARASITE COEVOLUTION

In nature, parasites are ubiquitous (Poulin 2007). Parasites[1] '*are organisms that live in, or on another organism, extract part or all of their organic nutrients from and cause damage to their hosts*' (Poulin 2007; Schmid-Hempel 2011). Parasites are so successful that they surpass other abundant and speciose groups such as birds in biomass (Kuris et al. 2008) and diversity (Windsor 1998). Parasitism evolved several times independently (Poulin 2007; Schmid-Hempel 2011). Parasites play a crucial role in population control as, for instance, in the case of the gastrointestinal nematode *Trichostringylus tenuis*, which impacts population cycles of red grouse (Figure 2A, Cattadori et al. 2005). Parasites link trophic levels within and among food webs and cycle nutrients as shown for the hemiparasitic mistletoe (Figure 2B, March and Watson 2010). By modulating predation risk and intraspecific competition, parasites impact ecosystem dynamics and stability (Figure 2C, reviewed in Hudson et al. 2006). It is because of this realized importance and complexity that research on host-parasite coevolution requires a multidisciplinary approach, employing

[1] For the purpose of this thesis we will focus on macro-parasites if not specified differently

knowledge from theoretical and empirical evolutionary biology, medicine and conservation and management of wildlife.

In addition, parasites have been identified as a selective agent strong enough to promote local adaptation (Kawecki and Ebert 2004; Greischar and Koskella 2007), reinforce population divergence (Figure 2D, MacColl 2009; Eizaguirre et al. 2011, 2012*a*) and fuel speciation in the host (Eizaguirre et al. 2009*a*; Feulner et al. 2015; Karvonen et al. 2015; Nagar and MacColl 2016). This is because locally distinct parasite communities select for counter-adaptations in the host to prevent infection, tissue damage and behavioural modifications. Such counter-adaptations include avoidance, tolerance or resistance (Ferrari et al. 2001; Raberg et al. 2007; De Roode and Lefèvre 2012); each a costly host defense mechanism (Sheldon and Verhulst 1996). Particularly, the evolution of resistance can engage the host and parasite in a coevolutionary struggle for dominance (Schmid-Hempel 2003).

Nevertheless, genetic drift and directional selection on resistance traits lead to a reduction in genetic diversity (e.g. Kimura 1968; Nurmonsky et al. 1998). Genetic variation, however, is a prerequisite to allow host populations to counter-adapt to parasite-mediated selection (King and Lively 2012). This is because specific host-genotype by parasite-genotype interactions determine host resistance, as postulated by the matching allele model (Howard and Lively 1994). Theoretically this results in an inverse relationship between infection rate and diversity in host genotypes (Lively 2010*a*). Parasite-mediated balancing selection should thereby maintain genetic variation (Haldane 1949). Experimental evidence corroborates this where antagonistic coevolution between the Red Flour Beetle *Tribolium castaneum* and its microsporidian parasite *Nosema whitei* leads to higher heterozygosity and allelic diversity in the host than owing to drift alone (Bérénos et al. 2011).

Two major evolutionary mechanisms are proposed to drive these coevolutionary dynamics of adaptation and counter-adaptation between host and parasite and maintain genetic variation: recurrent selective sweeps and frequency-dependent selection (Woolhouse et al. 2002; Brockhurst and Koskella 2013). These two dynamics assume a selective advantage of rare genotypes, but differ

in the way new genetic variants arise and spread within the population (Figure 3). Whereas recurrent selective sweeps are characterized by successive and often rapid fixation of *de novo* mutations or introgressed genes (Figure 3A, Buckling and Rainey 2002; Wegner and Eizaguirre 2012; Gokhale et al. 2013), frequency-dependent selection selects for genotypes present at low frequency in each population (Figure 3B, Van Valen 1973; Decaestecker et al. 2007; Hiltunen and Becks 2014). For instance, in the process of coevolution between *Chlorella variabilis* and its lytic dsDNA virus (Chlorovirus strain PBCV-1), the alga host rapidly evolves a generally resistant genotype following a period of recurring selective sweeps (Frickel et al. 2016). Typical negative frequency dependent selection, on the other hand, was observed between the freshwater snail (*Potamopyrgus antipodarum*) and its sterilizing trematode, *Microphallus* sp.: Under experimental coevolution between host and parasite the initially most common snail genotype decreases in frequency and becomes susceptible over the course of six generations (Koskella and Lively 2009). Neither selective sweeps nor frequency-dependent selection are mutually exclusive. However, they are not the only drivers of coevolutionary dynamics.

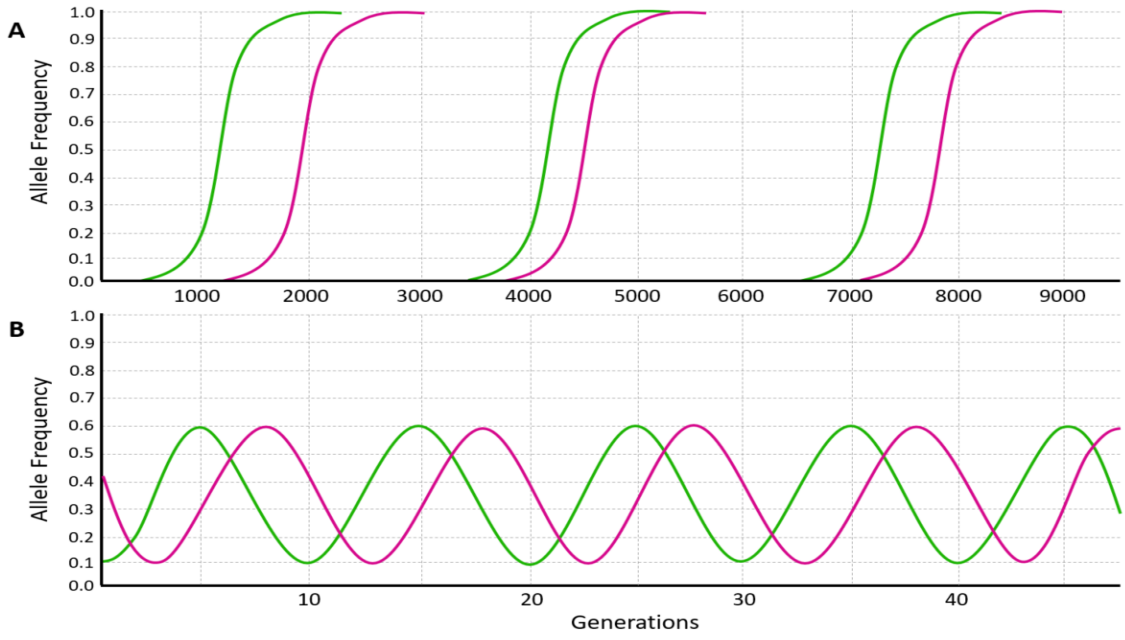

**Figure 3. Allele frequency changes driven by coevolutionary dynamics: A) recurrent selective sweeps by host (green) and parasite (pink) alleles fix *de-novo* mutations fast; B) dynamic oscillation in frequency of host (green) and parasite (pink) alleles causes a pattern of continuous cycling of rare and common variants. adapted from (Woolhouse et al. 2002).**

## THE ROLE OF SEXUAL SELECTION IN THE EVOLUTION OF HOST RESISTANCE

Besides mechanisms based purely on natural selection, sexual selection plays a role in the maintenance of genetic diversity (Andersson 1994; Milinski 2006) and evolution of resistance (Hamilton and Zuk 1982; Howard and Lively 1994). The importance of sexual selection emerges as a consequence of sexual reproduction. During meiosis, independent assortment and crossing over produces genetically variable haploid cells. Random fertilization between haploid cells then create novel combinations of genes and epistatic interactions (Maynard Smith 1978). But herein lies the crux because sexual reproduction requires the contribution of both sexes to produce a single offspring (Box 1). In the meantime, asexuals produce twice as many genetically identical copies. How can we explain the persistence of sex in spite of such a disadvantage? Asexual lines are thought to eventually degenerate owing to the accumulation of slightly deleterious mutations in the germline in each generation (i.e. Muller's Ratchet, Muller 1964). Sexual reproduction can purge such mutation. However, Muller's Ratchet was proposed to be too slow and weak to compensate for the 'two-fold disadvantage of sex'. Only under the additional assumptions of a higher mutation rate and epistatic interactions, the idea of mutation clearance as a function of sex could be rescued (Kondrashov 1988), although experimental evidence remains outstanding (but see Moya et al. 2004 for suggestive evidence).

**Box 1. On the 'two-fold disadvantage of sex'**

The maintenance of sex remains an unresolved evolutionary puzzle (Trivers 1985). Sexual reproduction seems wasteful given that sexual females first give up half of their genome during the process of meiosis and then produce both a son and a daughter (Figure 4). Since only daughters will again produce offspring but sons come at the same cost as daughters, sons seem an evolutionary waste. At the same time, an asexual female will have produced two identical daughters, each themselves giving rise to ever more offspring with the same genetic make-up of their asexual grandmother, soon to outcompete any sexual line (Maynard Smith 1978). And yet, sexual reproduction is common in nature (Kokko 2017) and found amongst parasites and hosts.

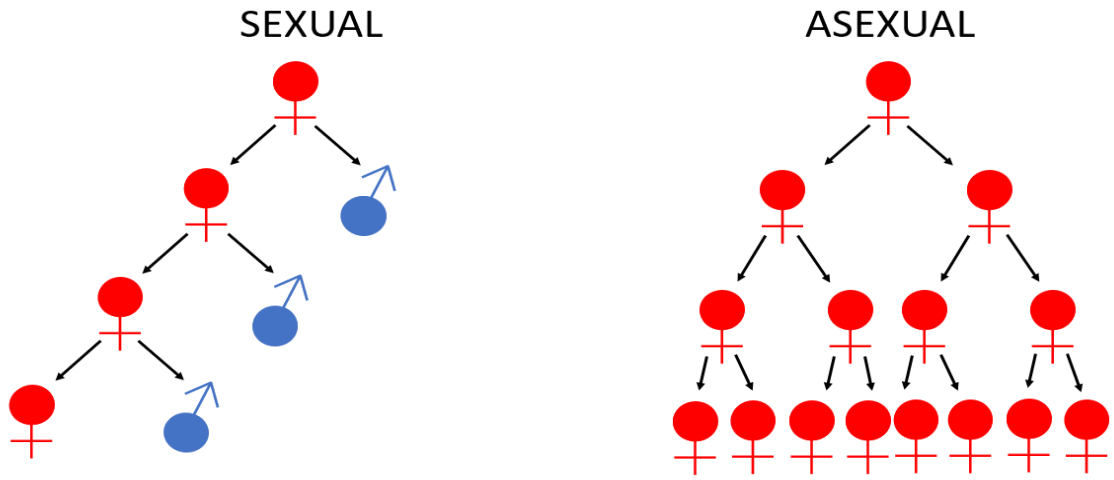


**Figure 4. The 'two-fold disadvantage of sex'. Redrawn from Maynard Smith 1978.**

The textbook explanation for the existence of sex proposes that sexual organisms are able to adapt more quickly to environmental change than asexuals can (Crow and Kimura 1965), given that sexuals reshuffle and recombine genetic material and generate potentially advantageous combinations in each generation (Fisher 1930; Milinski 2006). But already Maynard Smith noted that the environment change required to maintain an advantage of sex would mean '*that the correlations between selectively relevant features of the environment change sign between generations*' (Maynard

Smith 1978). Pathogens are features of the environment that can change sign between generations (Milinski 2006). According to the parasite Red Queen hypothesis (Van Valen 1973; Hamilton 1980), rare genes for resistance are favoured in every generation to deal with the changes in parasite community. Evidence for this comes from the snail-trematode system where sexual reproduction in the host, as shown by the presence of males, occurred more often with increasing trematode infection in a population (Lively 1987). Similarly, sexual topminnows (*Poeciliopsis monacha*) transplanted to freshwater ponds with clonal hybrids from *P. monacha* and *P. lucida* evolved resistance to infection by the trematode larvae (*Uvulifer* sp.) within two years, while the parasite load of the asexuals increased (Lively et al. 1990). The most recent evidence was gathered using the facultative sexual nematode *Caenorhabditis elegans* and strains of the bacterial pathogen, *Serratia marcescens* (Morran et al. 2011). The authors demonstrated that the frequency of sex rapidly increased from 20% to roughly 85% in the presence of the coevolving parasite. In addition, using host mutants that were either obligate sexuals or obligate selfing individuals, they showed that all asexual lines became extinct within 20 generations of coevolution (Morran et al. 2011).

In sexual organisms the two sexes also differ in their reproductive investment and strategies: females produce few, large gametes, whereas males produce many, small sperm. This anisogamy leads to females selecting their mates carefully and males competing to access females (Andersson 1994). The struggle to increase reproductive success was termed sexual selection with intersexual mate choice and intrasexual competition as its main mechanisms (Darwin 1871; Andersson 1994). Since the advantage of sexual reproduction relies on the reshuffling of genetic material in every generation, the advantage of mate choice, in particular, emerges when genetic variation amongst potential mates is large and an evolved mechanism exists to recognize one's own and that of the potential mates' genetic makeup in order to make an informed decision (Milinski 2006). This means that females can adjust to the ever-changing parasite community by identifying resistant mates

(Milinski 2006, 2016), potentially accelerating the evolution of resistant phenotypes (Eizaguirre et al. 2009*a*).

Mate choice is widespread in the animal kingdom (Andersson 1994). There are a variety of mechanisms underlying mate choice behaviour (Box 2), but for the purpose of this thesis we will mainly focus on those derived from indirect genetic benefits (Andersson and Simmons 2006; Puurtinen et al. 2009). Females use male signals for their mating decision in order to mate with high-quality males (Andersson 1994). Signals can be secondary sexual ornaments such as plumage colouration or courtship behaviours (Zahavi 1975; Hamilton and Zuk 1982). They relay information about the bearers' genetic quality, such as parasite resistance: this was found to be the case in North American passerines where male plumage brightness and song quality declined with increased parasite infection (Hamilton and Zuk 1982). The study concluded that females '*choose mates for genetic disease resistance by scrutiny of characters whose full expression is dependent on health and vigor*' (Hamilton and Zuk 1982) and many studies since support these findings (e.g. Milinski and Bakker 1990; Jennions et al. 2001; Faivre et al. 2003).

There are two modes of mate choice for indirect genetic benefits. Condition-dependent signals are thought to express additive genetic benefits, termed good genes, which increase individual fitness independent of genetic background (Puurtinen et al. 2009; Kuijper et al. 2012). Good genes reflect high fitness under current environmental conditions and may provide such benefits to the next generation under similar selection pressure. Another mechanism by which indirect genetic benefits emerge is through mate choice for compatible genes. Compatibility increases offspring fitness as a result of the combination of parental genes (Puurtinen et al. 2009). Choice for compatibility requires, however, both self-reference and a way to discern among mates (Milinski 2006).

Regardless whether mate choice is based on good genes or compatible genes, mate choice based on genetically-encoded resistance traits can contribute to the evolution of parasite resistance.

As such, and similar to natural selection, mate choice can lead to local adaptation (Eizaguirre et al. 2011; Andreou et al. 2017), accelerate host-parasite coevolution (Eizaguirre et al. 2012*b*) and speciation (Eizaguirre et al. 2009*a*; Maan and Seehausen 2011). Parasite-mediated sexual selection has been invoked to resolve the conundrum around the evolution and maintenance of sex, if the production of relatively fitter offspring compensates for the two-fold costs of sex (Maynard Smith 1978; Hamilton 1980; Trivers 1985; Milinski 2006; Jokela et al. 2009).

**Box 2. Mechanisms of Mate Choice**

a) *Inbreeding avoidance*: in species with high probability of inbreeding, mate choice may have evolved to allow kin discrimination and reduce inbreeding-associated costs (Jordan and Bruford 1998).

b) *Direct benefits:* female choice for males that provide direct benefits such as parental care, nutrition or access to high-quality territories (Møller and Jennions 2001).

c) *Sensory bias*: males evolve traits to increase their attractiveness to females owing to a preexisting perceptual biases (Ryan and Cummings 2013).

d) *Fisherian run-away*: genetic coupling between female preference and male trait expression leads to self-reinforcing run-away dynamics (Kirkpatrick 1982).

e) *Good genes*: secondary sexual traits reflect broad genetic quality such as resistance to infections (Zahavi 1975; Hamilton and Zuk 1982). Inherited genetic benefits remain stable if parents and offspring are exposed to similar environmental pressures (Kokko et al. 2003).

f) *Genetic Compatibility*: mate choice for compatible genetic make-up that combined with the genome of the chooser lead to higher fitness in the offspring (Tregenza and Wedell 2000; Aeschlimann et al. 2003).

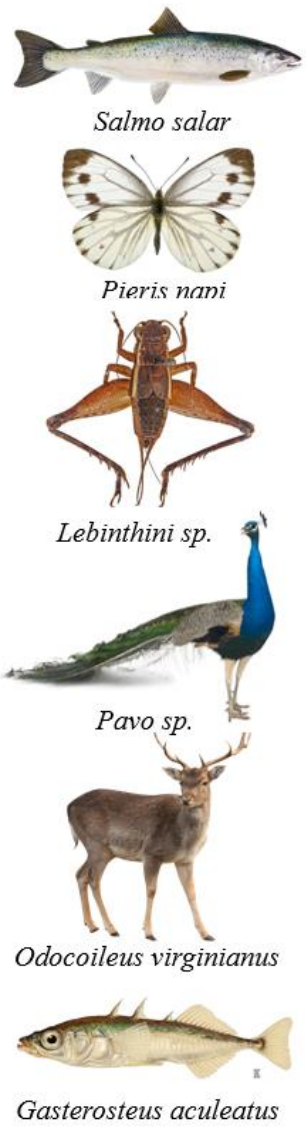


**Figure 5. Species with different mating mechanisms. From the top: Atlantic Salmon (*Salmo salar*, Landry et al. 2001); Green-veined White (*Pieris napi*, Karlsson 1998); Crickets from the tribe *Lebinthini* sp. (Ter Hofstede et al. 2015); birds from the genus *Pavo* sp. (Fisherian runaway envisioned only as thought experiment without experimental evidence); white-tailed deer (*Odocoileus virginianus*, Ditchkoff et al. 2001); three-spined stickleback (*G. aculeatus*, Milinski et al. 2005).**

## COEVOLUTION UNDER CONCOMITANT SELECTION PRESSURES

Beyond the intercept between parasite-mediated natural and sexual selection, the study of host-parasite coevolution is complicated by the fact that parasites are only one of many selective agents in nature (Figure 6; Betts et al. 2016). As part of any environment, hosts and parasites coevolve under concomitant selection by abiotic factors, such as temperature or precipitation (Lazzaro and Little 2009; Brunner and Eizaguirre 2016). Rising temperature, for instance, can be associated with increased parasite virulence or transmission, as observed for the cestode *S. solidus* which grows more quickly in its vertebrate host at higher temperatures (MacNab and Barber 2012). Temperature and humidity also impact transmission of the nematode *Ostertagia ostertagi* to its sheep host (*Ovis aries,* Stromberg 1997). From a host's perspective, a temperature rise from 15 to 25 degrees Celsius across seasons can alter susceptibility to infection among distinct *Daphnia* host genotypes (Mitchell et al. 2005). In three-spined stickleback, immunity (Dittmar et al. 2014) and survival under concomitant parasite exposure (Wegner et al. 2008) differ when stressed by heatwaves. And parasite exposure at distinct levels of eutrophication can impact stickleback populations with variable evolved resistance in contrasting ways, imposing distinct selection pressure on their prey community and future generations (Brunner et al. 2017). As such, environmental factors play a crucial role in the interaction between host and parasite (Wolinska and King 2009) and can even impact parasite-mediated speciation (Brunner and Eizaguirre 2016).

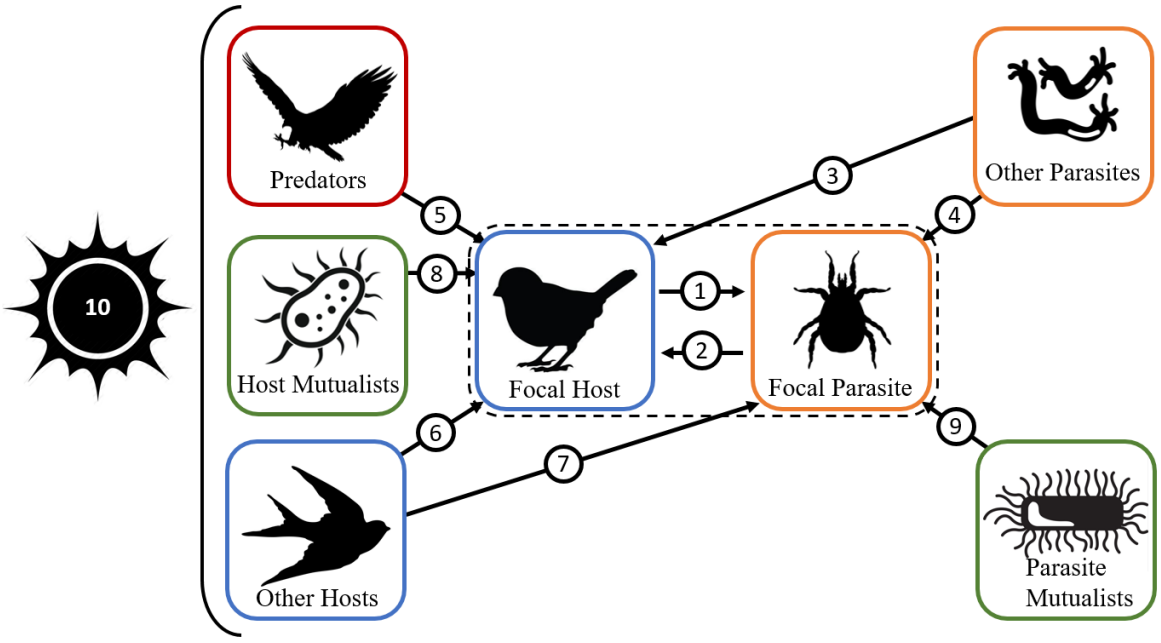


**Figure 6. The relationship between host (1) and parasite (2) can be altered by coinfection with additional parasite species (3,4). Host diversity can alter dynamics between host and parasite (6,7). Both predators (5) and defensive mutualists (8,9) can cause changes in the relationship. The biotic community as a whole is also impacted by changes in the abiotic environment (10).**

The diversity in hosts and parasites, whether from the same species or different species, also impacts host-parasite interactions (Figure 6). Coinfection can alter other parasite species' virulence, transmission, infective stage and host manipulation behaviour (Johnson and Hoverman 2012). For instance, when a copepod host is infected with either the cestode *S. solidus* or the nematode *Camallanus lacustris*, the infective parasite will start manipulating host behaviour. When both grow inside the same host the infective parasite will suppress the other's development (Hafer and Milinski 2016). In other cases, infection by one parasite favours coinfection by others (Benesh and Kalbe 2016). The diversity of different host species, or the genetic diversity within a species, shapes parasite communities (Carius et al. 2001; Woolhouse et al. 2002). A decline in rodent diversity, for example, increased the prevalence of Lyme-disease-bearing ticks (LoGiudice et al. 2003) and inbred

populations of Californian sea lion *Zalophus californianus* experience higher helminth infection and disease susceptibility (Acevedo-Whitehouse et al. 2003).

Selection by other biotic factors can interfere with host-parasite coevolution (Figure 6). Ecological feedbacks between prey-predator and host-parasites dynamics can lead theoretically to changes in parasite infection and transmission (Poulin 2007; Best 2018). For instance, the presence of the herbivorous Oleander aphid (*Aphis nerii*) decreases cardenolide concentration, a defensive chemical employed by milkweed plants to fend off herbivorous monarch butterfly (*Danaus plexippus*) caterpillars, but indirectly increases the virulence and transmission potential of the monarch butterfly's protozoan parasite *Ophryocystis elektroscirrha* (De Roode et al. 2011). Vice versa, host manipulation by parasites can increase predation susceptibility as seen in killifish (*F. parvipinnis*, Lafferty and Morris 1996) or red grouse (*L. lagopus scoticus*, Hudson and Dobson 1992). In three-spined sticklebacks from Roberts lake, acquired resistance against a cestode comes at the extreme cost of practically sterilizing females (Weber et al. 2017), but resistance likely reduces predation risk at the same time. Such trade-offs in adaptation between parasite and predator-mediated selection may lead to the breakdown of arms race dynamics, as observed in the coevolution experiment between the bacteria *Pseudomonas fluorescens*, its obligate SBW25Φ2 bacteriophage and the predatory ciliate *Tetrahymena thermophila* (Friman and Buckling 2013).

How can hosts evolve resistance to parasites faced with such a diversity of concomitant selection pressures? This question is largely unexplored. It remains difficult to design evolutionary experiments complex and powerful enough to tease dynamics apart (Pérez-Jvostov et al. 2012). But more comprehensive experimental approaches, such as mesocosm experiments, start to unravel the intricate interactions in even simple communities (e.g. Brunner et al. 2017). But given the complexity of ecological interactions and competing selection pressure the gap is likely bigger than anticipated. Previously host-parasite coevolution has been studied in isolation, but with this thesis we are

attempting to explore coevolution and, in particular, the evolution of host resistance in more complex scenarios.

**Host resistance evolution**

INNATE AND ADAPTIVE IMMUNITY IN VERTEBRATES

The capacity to generate non-specific immune responses against foreign invaders is found in all multicellular organisms (Medzhitov and Janeway 1997; Hoffmann et al. 1999). Innate immunity relies on the ability of germline-encoded pattern-recognition receptors (PRRs) to differentiate conserved pathogen-associated molecular patterns from noninfectious products synthesized by the host itself (Janeway and Medzhitov 2002; Janeway et al. 2005). Pathogen recognition and initiation of immune responses are mediated by Toll-like receptors (TLRs, Medzhitov and Janeway 1997), which, contrary to previous assumptions, can be polymorphic (Tschirren et al. 2013). In vertebrates, TLRs initiate innate immune responses by binding ligands to leukocytes' membranes and T and B cells, triggering the activation of the adaptive immunity (Medzhitov and Janeway 1997; Takeda and Akira 2005). Innate immune responses, while rapid, are limited and less potent than responses stemming from the adaptive immunity found in jawed vertebrates (Table 1, Janeway 2005).

Adaptive immunity responds to specific infections using clonally expressed receptors generated somatically and with seemingly limitless specificity (Figure 7, Janeway et al. 2005). The pathogen specificity maximizes the efficacy of the immune reaction, while limiting immunopathological costs associated with non-specific responses (Janeway et al. 2005; Palm and Medzhitov 2009). The adaptive immune system furthermore has the capacity to rapidly re-identify pathogens experienced previously (Table 1, Medzhitov and Janeway 1997; Palm and Medzhitov 2009). This is because of clonal expression and selection, T and B memory cells of the adaptive immune systems aid in retaining information about previous infections, conferring long-term

protection against future re-infections (Ahmed and Gray 1995; Palm and Medzhitov 2009). Similar to TLRs, the peptide-binding molecular structures encoded by genes of the Major Histocompatibility Complex (MHC) are instrumental to pathogen recognition and elimination by T cells (Janeway et al. 2005). Primary activation of adaptive immune system, however, requires substantially more time compared to innate immune responses. In addition, randomly generated antigen receptors can also initiate responses against self-antigens or innocuous non-self-antigens, leading to autoimmunity or allergies, respectively (Janeway et al. 2005; Palm and Medzhitov 2009).

**Table 1. Comparing the innate and adaptive immunity. Descriptions from Janeway 2005.**

| **CHARACTERISTICS** | **Innate** | **Adaptive** |
|---|---|---|
| Reaction time | Rapid upon initial contact | Slow primary immune response, rapid secondary initiation |
| Specificity | Recognizes conserved pathogen associated molecular patterns | Recognizes microbial and non-microbial antigens |
| Diversity | Limited in scope; germline encoded | Exceptionally large; receptors are clonally generated by somatic recombination |
| Memory | None (but see Kurtz and Franz 2003) | Yes via T and B memory cells |
| Non-reactivity to self | PRRs respond only to pathogen-associated molecular patterns | can respond to self-antigens and innocuous non-self-antigens |

## THE MAJOR HISTOCOMPATIBILITY COMPLEX

In vertebrates, genes of the Major Histocompatibility Complex (MHC; human leukocyte antigen (HLA) in humans) are the best described genetic basis of parasite resistance (Klein and Figueroa 1986). As part of the adaptive immune system, this highly polymorphic, gene dense genomic

region encodes for various immunologically important cell surface proteins (Apanius et al. 1997). Specifically, MHC molecules function as a peptide shuttle that transport peptides from the cytoplasm to be presented on the cell surface (Janeway et al. 2005). Classical MHC molecules are divided into MHC class I and class II. While both present self- and foreign-peptides on the cell surface, Class I molecules derive foreign peptides from proteins broken down by proteasome and present them to CD8+ cytotoxic T cells. MHC class II molecules, on the other hand, display self-peptides and endocytosed extracellular parasite-derived antigens to the cell surface to be bound by CD4+ T helper cells. In both cases, antigenic peptides are anchored at antigen-binding sites. Foreign antigens will then trigger a highly specific immune reaction, while self-antigens are tolerated (Janeway et al. 2005). Immune activation will also culminate in the establishment of immunological memory via T memory and B memory cells, which circulate at low frequency in the host's bloodstream even after the infection is eliminated, but are quick to recognize recurring parasites and initiate fast immune responses thereafter (Figure 7, Ahmed and Gray 1995). MHC genes are therefore vital in the detection and elimination of pathogens, including extra-cellular macroparasites, via adaptive immunity.

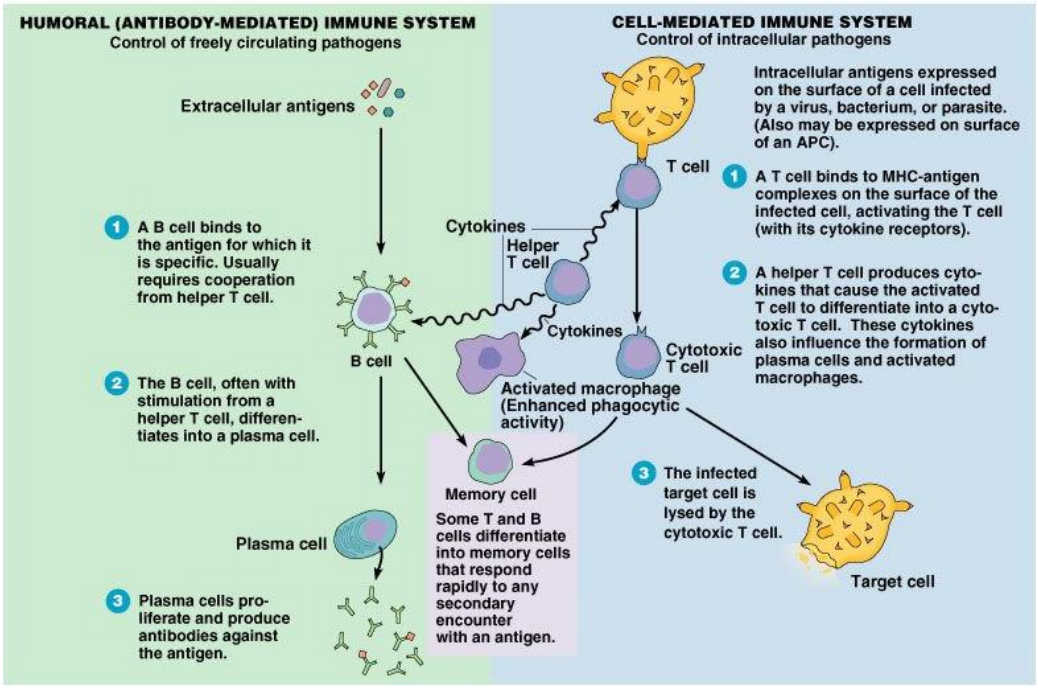

**Figure 7. Description of both hormonal and cell-mediated immune systems in jawed vertebrates. Redrawn from [https://mysullys.com/cell-mediated-immune-response-flow-chart/immune-response-cell-mediated-humoral-immunity-531815544684/](https://mysullys.com/cell-mediated-immune-response-flow-chart/immune-response-cell-mediated-humoral-immunity-531815544684/);**

NATURAL AND SEXUAL SELECTION ON THE MHC

The exceptional polymorphism, both in terms of allele and sequence divergence, is a key feature of MHC genes in jawed vertebrates (Apanius et al. 1997), including for example some salmonids (Aguilar and Garza 2007), great reed warblers (*Acrocephalus arundinaceus*, Westerdahl et al. 2004), Soay sheep (*O. aries*, Charbonnel and Pemberton 2005) and humans (*Homo sapiens*, Hedrick and Thomson 1983). This diversity of MHC genes is maintained by balancing selection (Hedrick 1994). Several processes such as selection for specific rare and especially advantageous alleles, selection on MHC allele diversity or between populations with distinct MHC allele pools interact to preserve a diverse MHC allele repertoire within and among populations (Figure 8, reviewed in Eizaguirre and Lenz 2010). In addition, sexual selection and specifically assortative mate choice between populations, and for compatible genes and good genes, play an important role in the maintenance of MHC polymorphism (Figure 9, Eizaguirre et al. 2009*b*; Milinski 2014). Here we will review the current knowledge about their relative contribution:

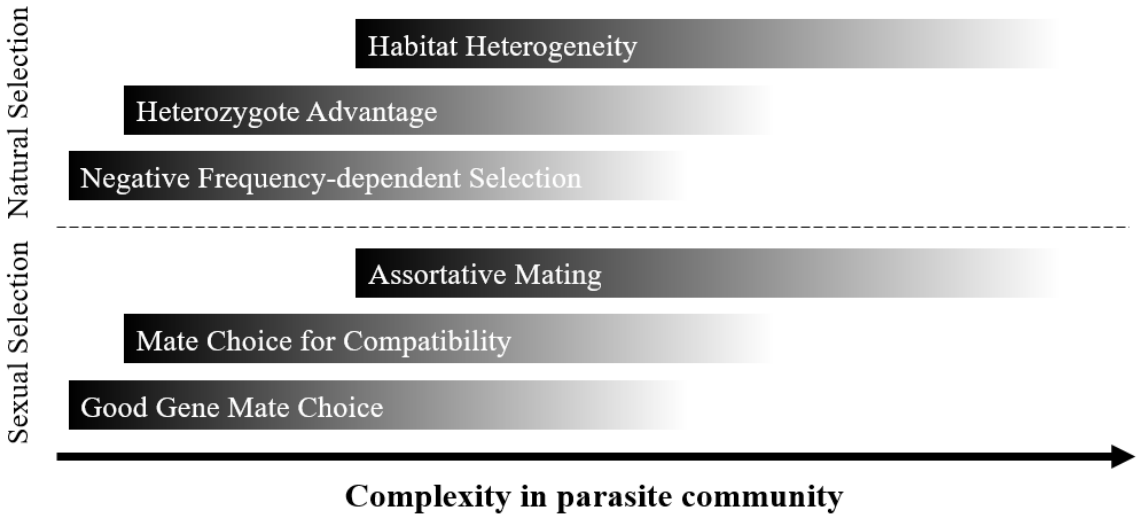


**Figure 8. Parasite-mediated selection maintains polymorphism at the major histocompatibility**

**complex. Distinct selection mechanisms act at different scales of complexity in parasite diversity and spatial or temporal distribution of parasites. Negative frequency-dependent selection describes the interaction between individual MHC alleles and specific parasite genotypes and species at low complexity. Similarly, mate choice based on good genes reinforces selection on specific advantageous MHC alleles that currently provide resistance against a specific parasite. Heterozygote advantage (or optimal MHC diversity) is the resistance benefit of individual MHC diversity with increasing genetic diversity of parasites. Mate choice for compatible genes maintains offspring with an optimal individual MHC diversity to resist a more diverse parasite community. Habitat heterogeneity increases the likelihood of occurrence and spatial or temporal distribution of species across more diverse environments and, hence, maintains a diverse MHC allele pool. Population-specific assortative mating becomes important under higher parasite complexity across habitats as to maintain MHC allele diversity across habitats.** Figure modified from Eizaguirre & Lenz 2010.

*Negative frequency-dependent selection (NFDS).* At a population level, frequency-dependent coevolutionary dynamics between host and parasite genotypes are important to maintain allelic diversity at the MHC. The advantage of rare host genotypes relies on the parasite adapting to the most common host genotype (Dybdahl and Lively 1998; Decaestecker et al. 2007). Accordingly, rare MHC alleles are more likely to be resistant against common parasites and, hence, confer a selective advantage (Ejsmond and Radwan 2015). But since parasites counter-adapt once specific MHC alleles rise in frequency, NFDS will lead to high allelic turnover, avoiding both fixation and loss of alleles in the process (Figure 4B, Takahata and Nei 1990). Despite this clear theoretical underpinning, negative frequency-dependent selection for MHC genes is only supported by a handful of studies and often via inferences: Westerdahl and colleagues (2004) compared variation in 23 MHC class I alleles with the variability in neutral microsatellite markers in great reed warblers (*A. arundinaceus*) for nine generations. They found that variation in MHC allele frequencies was greater than for neutrally evolving markers, as predicted by NFDS. Similarly, in a 13 year long survey, Charbonnel and Pemberton (2005) showed that the genetic differentiation was higher at the MHC class II site than at neural loci for Soay sheep (*O. aries*), which are heavily afflicted by the nematode *Teladorsagia*

*circumcincta*. In another case, a specific MHC class II*β* allele provided resistance against a prevalent ectoparasite in a population of three-spined stickleback (*G. aculeatus*) in one year, but no longer provided resistance against the monogenean ectoparasite in the following year, albeit having increased in frequency. This suggests rapid counter-adaptation by the parasite (Eizaguirre et al. 2009*b*; Lenz et al. 2009*b*). Rapid adaptation by the stickleback host was later proven experimentally in a transgenerational experiment where half of the six populations were exposed to either *Anguillicola crassus* or *C. lacustris* nematode parasites. The authors were able to show a rapid shift in MHC allele frequency between generations in favour of alleles that increased resistance to the exposed parasite (Figure 9, Eizaguirre et al. 2012*b*). These studies demonstrate adaptive shifts in MHC alleles, as proposed by NFDS. Evidence for enhanced resistance of individuals with rare MHC alleles, however, is still scarce. Recent work on the stickleback population from Roberts Lake (British Columbia) showed some evidence of resistance advantages of individuals with rare, i.e. "immigrant", MHC alleles when transplanted between lake and the adjacent river (Bolnick and Stutz 2017), despite this contradicting divergent parasite-mediated selection between fish from river and lake generally (Eizaguirre et al. 2012*a*; Kaufmann et al. 2017). In the Trinidadian guppy (*Poecilia reticulata*), novel, i.e. introgressed, MHC variants also seem to provide resistance under laboratory settings (Phillips et al. 2018) in line with theoretical predictions (Ejsmond and Radwan 2015). Collectively these studies highlight that (i) MHC alleles change across generations and (ii) the selective advantage of specific MHC alleles predicts frequency changes. These findings are compelling proof for a role of NFDS in the maintenance of MHC diversity.

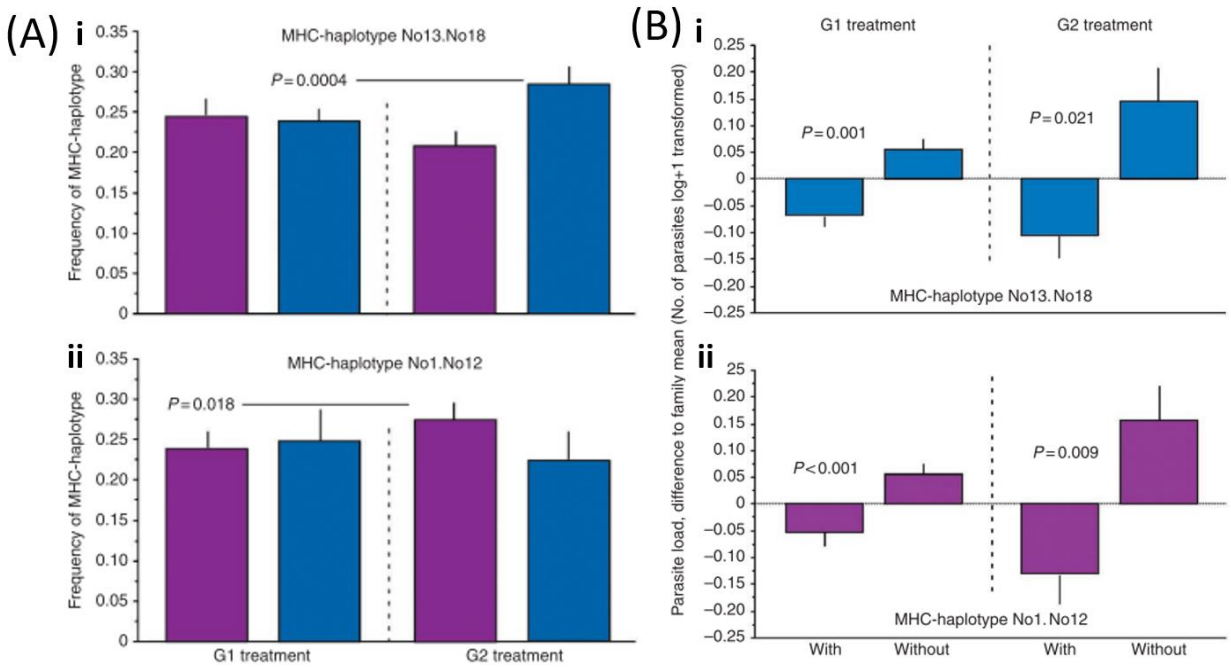


**Figure 9. A i + ii) the frequency of those MHC alleles providing resistance against the exposed parasite increased in the G2 generation. B i + ii) parasite specific resistance advantages of MHC alleles is maintained across generations. Redrawn from Eizaguirre et al. 2012b.**

*Heterozygote/divergent allele advantage*. While NFDS favours individual and highly advantageous MHC alleles, MHC allele diversity is likely also under positive selection. Distinct MHC-based resistance arises due to structural differences in the peptide-binding grove amongst MHC-encoded molecules (Janeway et al. 2005). A great variety of MHC molecules is encoded by a diverse set of codominant expressed MHC alleles, which consequently binds and detects a wider array of pathogen antigens (Doherty and Zinkernagel 1975). The excess of non-synonymous over synonymous nucleotide substitutions (Hughes and Nei 1988) and the large sequence divergence amongst alleles (Klein et al. 2007) support arguments for positive selection on MHC allele diversity. This suggests MHC heterozygous individuals are fitter than MHC homozygous individuals (Figure 10, Doherty and Zinkernagel 1975; Pitcher and Neff 2006). Yet, support for the heterozygote hypothesis is mixed: MHC heterozygous water voles (*Arvicola terrestris*), for instance, had a lower parasite load than MHC homozygous individuals (Oliver et al. 2009). By contrast, heterozygote

crosses between wild and laboratory-bred mice displayed lower resistance, reduced reproductive success and survival (Ilmonen et al. 2007). The heterozygote advantage hypothesis was later extended to include the idea that heterozygous individuals with higher allelic divergence gain increased resistance against multiple parasites (Wakeland et al. 1990). Atlantic Salmon (*Salmo salar*) with more divergent MHC alleles showed increased resistance to a single parasite species, lending some weight to the divergent allele advantage hypothesis (Consuegra and de Leaniz 2008). Similarly, higher number of MHC alleles and sequence divergence both raised resistance to helminth parasites and were associated with increased body condition of the Long-tailed giant rat (*Leopoldamys sabanus*, Lenz et al. 2009*c*). In grey seal *Halichoerus grypus,* the number of MHC alleles determined survival to adulthood (De Assunção-Franco et al. 2012). Furthermore, MHC allele divergence was negatively correlated with parasite load in river and lake-transplanted three-spined stickleback (Eizaguirre et al. 2012*a*). Yet, despite some empirical support for heterozygote/divergent allele advantage (reviewed in Eizaguirre and Lenz 2010), theoretical work suggests that the vast diversity found in MHC genes cannot be explained by heterozygote advantage alone (De Boer et al. 2004).

*Optimal heterozygosity.* Interestingly, intra-individual MHC diversity is typically limited to a subset of the entire allele pool of a population (Stet et al 2003), which is paradoxical since the mechanism that leads to high polymorphism within a populationdoes not lead to higher intra-individual MHC diversity. This is surprising given the potential for evolutionary duplication and diversification of individual loci (Lawlor et al. 1990). In fact, selection may favour an immunogenetic optimum number of MHC alleles over maximal MHC diversity in the case when an increased capacity for antigen recognition at high MHC diversity is offset by a deselection in T cell repertoire as to avoid autoimmunity, the so-called negative T cell selection (Box 3, Figure 10B, Relle and Schwarting 2012; Klein et al. 2014). This was first mathematically predicted (Nowak et al. 1992; Woelfing et al. 2009), and a high frequency of individuals carrying an intermediate number of MHC alleles is commonly found amongst natural populations, including three-spined sticklebacks (*G. aculeatus,* Reusch et al.

2001*b*), loggerhead sea turtles (*Caretta caretta*, Stiebens et al. 2013) and California sea lions (*Z. californianus*, Acevedo-Whitehouse et al. 2018). Furthermore, empirical evidence from several species now describes highest parasite resistance or fitness at an optimally intermediate individual MHC diversity (Figure 10B, e.g. three-spined stickleback, (Wegner et al. 2003*a*; Kalbe et al. 2009); blunt-head cichlids (Hablützel et al. 2014); loggerhead sea turtles (Stiebens et al. 2013); pythons (Madsen and Ujvari 2006); turkeys (Buchholz et al. 2004); bank voles (Kloch et al. 2010)).

**Box 3. T cell selection.** During the process called positive T cell selection newly formed T cell receptors are tested for reactivity to the individual's MHC-molecules and only those T cells with affinity for binding complex MHC-molecules will be retained (Janeway et al. 2005). As individual MHC diversity increases the number of positively selected T cell lines will rise, improving the likelihood of detecting infections with parasites or pathogens (Figure 13A, Doherty and Zinkernagel 1975). Following positive selection, T cells down-regulate the expression of one of the two co-receptors, CD4 and CD8, and morph into CD4+ or CD8+ single positive T cells. After transformation, T cells whose receptors respond too strongly to self-peptides are eliminated in a process called negative selection (Figure 10A). This is because self and non-self-discrimination are controlled by MHC molecules and the binding of self-peptides as foreign is hypothesized to result in immune activation and eventually auto-immune diseases (Germain 1994). This trade-off theoretically selects for an optimal rather than a maximal number of individual MHC alleles (Nowak et al. 1992; Woelfing et al. 2009), which was supported empirically (Figure 10B, Wegner et al. 2003*a*).

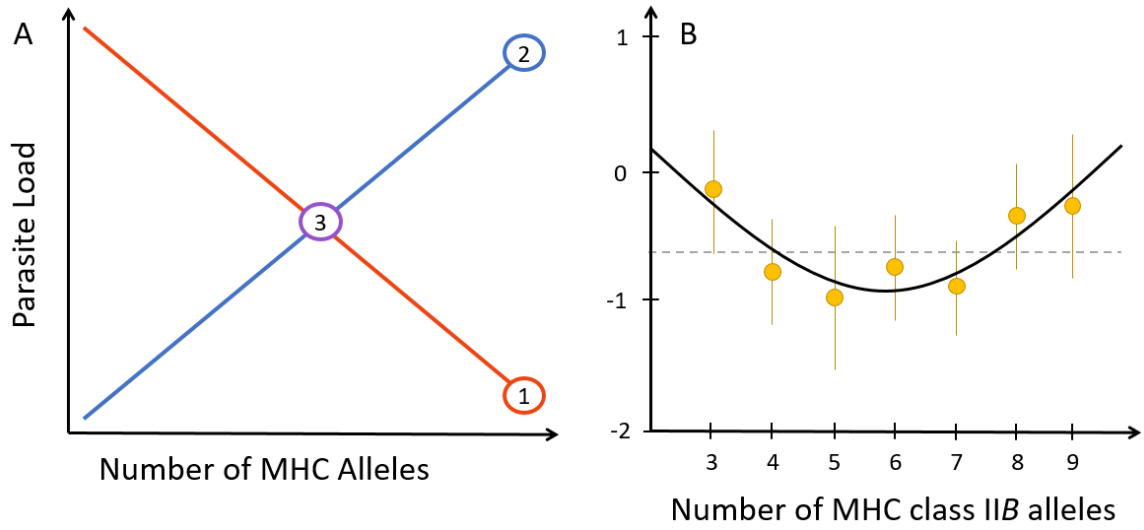


**Figure 10. A) The balance between (1) positive, i.e. increased resistance against a diverse set of parasites as consequence of increased individual MHC allele diversity, and (2) negative, i.e. increased deselection of T cells that react to strongly with self-peptides, selection leads to (3) an optimal number of different MHC alleles at which resistance towards parasites is maximised. B) This was first shown for three-spined stickleback, where the overall parasite load was lowest at intermediately high MHC class II*β* allele diversity. Figure redrawn from Wegner et al. 2003*a*.**

*Spatial and temporal heterogeneity.* Spatial variation in parasite-mediated selection is another mechanism by which MHC polymorphism is maintained. Specifically, locally adapted MHC allele pools contrast between different environments due to community and species differences in parasites (Figure 8, Thompson 1994; Kalbe et al. 2002). Likewise, temporal variation, such as differences between years and seasons can cause a change in parasite community (Nuismer et al. 2003). In both cases, this emerges based on different biotic and abiotic conditions. Evidence for this has been gathered from several neighboring populations that were found to differ in their MHC allele pools (e.g. greater snipe, (Ekblom et al. 2007); three-spined stickleback, (Wegner et al. 2003*b*); African cichlid, (Blais et al. 2007); eastern Atlantic grey seal, (Cammen et al. 2011)). In house sparrows (*Passer domesticus*), for example, MHC-mediated resistance to malaria was found to be population-specific (Bonneaud et al. 2006*b*). Divergent selection on the MHC repertoire between populations inhabiting environments of distinct parasite diversity may even result in population

divergence (Eizaguirre et al. 2012*a*), but, at a metapopulation level, it allows for the maintenance of a diverse MHC allele pool, increasing the potential for counter-adaptation against coevolving parasites.

*MHC-based mate choice.* In addition to parasite resistance, MHC is widely recognized as the best-known genetic basis for mate choice (Milinski 2006; Kamiya et al. 2014). In fact, mate choice based on MHC is found in a vast number of jawed vertebrates (e.g. salmon, (Landry et al. 2001); three-spined stickleback, (Milinski et al. 2005); great snipe, (Ekblom et al. 2004); mice, (Penn and Potts 1999); human, (Wedekind et al. 1995)), although the underlying mechanisms differ (Milinski 2015):

Studies in mice (Leinders-Zufall et al. 2004), fish (Milinski et al. 2005) and humans (Milinski et al. 2013) demonstrate that MHC peptides can function as olfactory cues that females can use to discern between mates. These MHC peptide ligaments are structural mirror images of the genetically encoded binding groove of the MHC molecule and appear in bodily fluids after they are liberated from the peptide-MHC complex (Milinski et al. 2005). This makes them available for olfactory assessment by females. Several studies have shown that these odour signals can be used to differentiate between relatives and ascribe MHC-based mate choice a role in inbreeding avoidance (Yamazaki et al. 1976; Potts et al. 1991). Yet, odour signals from the major urinary protein gene cluster are more likely involved in inbreeding avoidance (Sherborne et al. 2007). Regardless, differentiation between MHC peptide ligaments also raises the possibility of mate choice for compatible MHC alleles in order for females to complement their own set of genes, resulting in an optimal (i.e. either maximal or intermediate) MHC diversity for the offspring (Reusch et al. 2001*a*; Aeschlimann et al. 2003; Milinski et al. 2005). Breeding pair formation in house sparrows *P. domesticus*, for example, was positively correlated with MHC diversity, irrespective of relatedness (Bonneaud et al. 2006*a*). And human women employ olfactory cues to select the most MHC dissimilar partner when given a choice (Wedekind et al. 1995).

By contrast, specific MHC alleles can also function as good genes (Landry et al. 2001; Ekblom et al. 2004). Specific MHC alleles may confer resistance against currently prevalent parasite species and therefore benefit the host's fitness (Milinski and Bakker 1990). Such functional advantage allows males to display sexual signals (Hamilton and Zuk 1982), indicating their genetic quality honestly (Zahavi 1975). MHC-based good genes may benefit males in male to male combat, as suggested for white-tailed deer (*O. viriginianus* (Ditchkoff et al. 2001), and sexual selection via mate choice (Andersson 1994): breeding colouration in three-spined stickleback, *G. aculeatus*, was linked to a specific MHC Class I haplotype (Jäger et al. 2007) and snood length in wild turkeys, *Meleagris gallopavo*, to a MHC Class II*β* allele (Buchholz et al. 2004).

MHC-based mate choice for compatibility and good genes are not mutually exclusive (Eizaguirre et al. 2009*b*), but function via different sensory pathways: olfactory cues allow females to assess a mate's MHC compatibility from afar and closer inspection via visual cues help to determine whether males have specific beneficial MHC alleles (Jäger et al. 2007; Eizaguirre et al. 2009*b*). Both may in fact be complementary strategies. Moreover, they likely play different roles at different levels of parasite-mediated complexity, similar to mechanisms of natural selection (Figure 9): Unlike mate choice for specific good genes, mate choice for MHC compatibility distinguishes MHC alleles not based on the intrinsic quality of individual MHC alleles but selects based on the benefits from combining two sets of MHC alleles. This allows for rapid local adaptation to prevalent parasite species via good genes, while maintaining MHC polymorphism via choice for compatibility (Eizaguirre et al. 2009*b*; Milinski 2015). But whereas MHC-based mate choice maintains a diverse pool of MHC alleles at a population level, the same mechanism strengthens divergence between population by imposing another barrier against mixing between locally adapted MHC allele pools (similar to habitat heterogeneity; Figure 9, Eizaguirre et al. 2011; Andreou et al. 2017). Parasite-mediated MHC-based mate choice therefore promotes rapid, i.e. faster than natural selection alone, local adaptation, population divergence and speciation (Nuismer et al. 2008; Eizaguirre et al. 2009*a*).

*Maintenance of MHC polymorphism in spite of concomitant selection.* Crucially, it remains unclear how concomitant selection pressures other than parasite-mediated natural and sexual selection affect the maintenance of MHC diversity. Changes in host density, for instance, by predation (Pérez-Jvostov et al. 2012), could have the potential to alter sexual and natural selection dynamics via altering female choosiness or parasite transmission, respectively (Eizaguirre et al. 2009*b*). And resistance or the lack thereof may affect ecosystem or community dynamics by altering foraging behaviour of predators and prey (Milinski 1990; Anaya-Rojas et al. 2016). Yet, such complex interdependencies require more empirical tests.

**The Three-spined stickleback as model organism**

The three-spined stickleback (*Gasterosteus aculeatus*) is widely used as a model organism to tackle ecological, developmental and evolutionary questions (Peichel et al. 2001; Gibson 2005). This small teleost fish is abundant throughout marine and freshwater environments in the Northern Hemisphere and offers a traceable, rapidly evolving and easy to rear system (Peichel and Boughman 2003; Gibson 2005). Early work on stickleback has contributed towards our understanding of animal behaviour (Tinbergen 1951; Milinski and Heller 1978) and ecological speciation (Bell and Foster 1994; Schluter 1995). More recently the availability of molecular and genomic tools allowed for more detailed investigation of genomic and phenotypic divergence (Colosimo et al. 2005; Feulner et al. 2015; Marques et al. 2016). Moreover, short generation times in this model helped study its role in transgenerational dynamics (e.g. Matthews et al. 2016; Brunner et al. 2017) and rapid adaptation over short evolutionary time (e.g. Reusch et al. 2001*b*; Schmid et al. 2019), as well as dynamic predator-prey and host-parasite coevolution (e.g. Milinski 1987; Eizaguirre et al. 2012*a*).

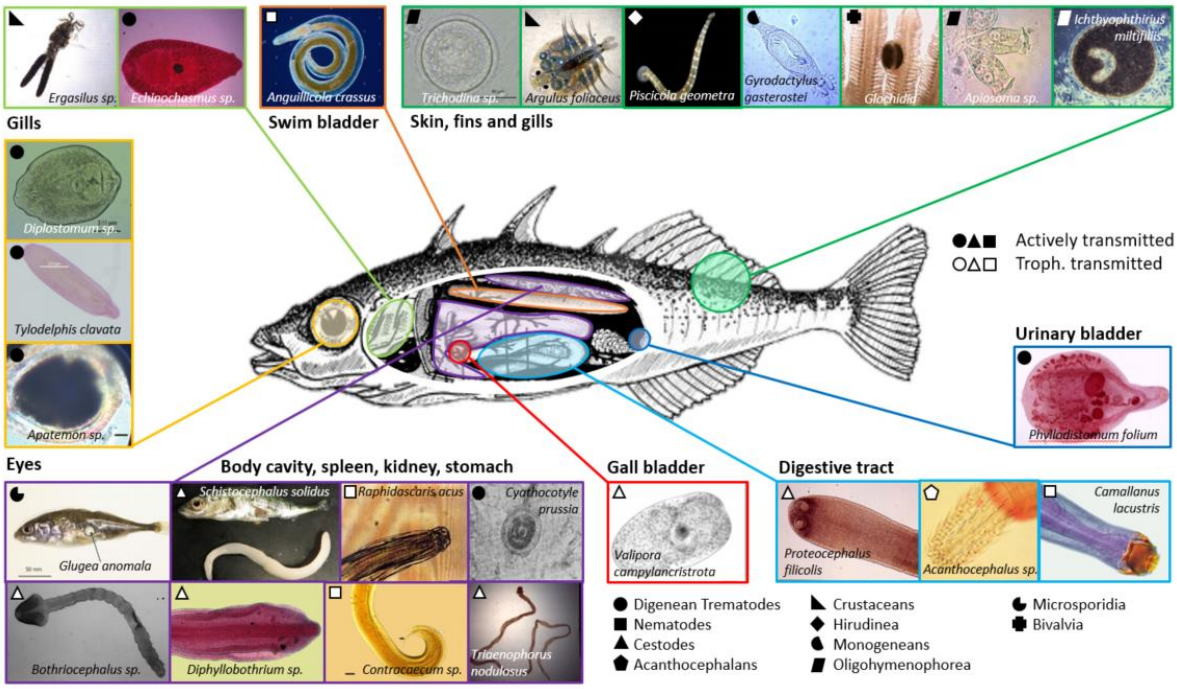


**Figure 11. A variety of parasites infect freshwater three-spined stickleback. Redrawn from M. Kalbe.**

BIOLOGY AND LIFE-HISTORY OF FRESHWATER THREE-SPINED STICKLEBACKS

Three-spined stickleback (*G. aculeatus*) are meso-predators in most of their range, feeding predominantly on zooplankton or/and benthic invertebrates (Schluter 1993; Lucek et al. 2012). They are preyed upon by both piscivorous fish and birds (Wootton 1976). The species can also be both final or intermediate hosts in a parasite's life cycle and acquires both, trophically and actively infection parasites throughout their life (Figure 11, Barber 2013; Stewart et al. 2017). The species is recognized as an important cornerstone organism in aquatic environments.

In most temperate environments, juvenile stickleback are born during late spring/early summer and grow until the next spring when they reproduce as adults (Wootton 1976). Adult stickleback will die soon after their first reproductive period. During the breeding season, male three-spined stickleback will establish territories, display a red throat colouration as a sexual signal and start building a nest out of organic or inorganic material (Wootton 1976). The fragile nest structure is

glued together by a glycoprotein named Spiggin synthesized from the hypertrophied kidney (Kawahara and Nishida 2006). The integrity of the nest structure is maintained by frequent glue secretion during "swim-through" and release on top of the nest (Wootton 1976). Upon approach by a gravid female, male three-spined stickleback will court her (often in form of a "zig-zag" dance, Tinbergen 1951) and lead her to the entrance of the nest. Female stickleback will inspect both the males' display and the nest structure (i.e. "nosing" behavior) before she spawns. After the male fertilizes the clutch, he will chase away the female and provide paternal care in form of egg fanning and clearance of molded eggs (Bell and Foster 1994). Yet, sneaking is a commonly reported alternative fertilization strategy of male sticklebacks, where males other than the nest owners steal fertilization without having to invest into nest construction and parental care (Largiader et al. 2001). Paternity and nest ownership can, however, be traced using parenthood analysis, allowing to assess the ultimate Darwinian measure of fitness, lifetime reproductive success (Kalbe et al. 2009).

## THE ROLE OF THE MHC FOR PARASITE RESISTANCE IN STICKLEBACK

Allelic diversity at the MHC region varies widely across populations (Reusch and Langefors 2005; Eizaguirre et al. 2011; Feulner et al. 2015) with evidence of divergent parasite-mediated selection on MHC class II*β* alleles (Eizaguirre et al. 2012*a*), supporting the idea of local adaptation driving population divergences between populations (Eizaguirre et al. 2009*a*). At a population level, parasite resistance is highest at an optimally intermediate number of MHC alleles (Wegner et al. 2003*a*) with consequences for survival (Wegner et al. 2008), immune functions (Kurtz et al. 2004) and reproduction (Kalbe et al. 2009). There is also evidence that individual MHC alleles provide increased resistance against specific parasites (Eizaguirre et al. 2009*b*; Kaufmann et al. 2017) and following that the frequency of such alleles increases rapidly (Eizaguirre et al. 2012*b*; Bolnick and Stutz 2017). Collectively, these results suggests maintenance of MHC polymorphism by habitat heterogeneity, heterozygote advantage and NFDS.

Female stickleback choose mates based on MHC-mediated good genes and olfactory cues for MHC compatibility (Milinski and Bakker 1990; Milinski et al. 2005). After establishing a territory and building a nest, males produce and distribute energetically costly MHC peptide ligands (Milinski et al. 2010) used by females to assess potential mates from a distance (Jäger et al. 2007). A female will seek males with whom, when combined with her own MHC alleles, she will achieve an optimally intermediate MHC diversity in a strategy to produce offspring close to the population-specific optimal MHC individual diversity (Milinski et al. 2005; Andreou et al. 2017). This means stickleback females use self-reference and odour signals from nesting males to find compatible mates (Aeschlimann et al. 2003; Milinski et al. 2005).

In addition, male size and carotenoid-based throat colouration communicate the parasite load and thus immunogenetic quality of the mate (Milinski and Bakker 1990; Jäger et al. 2007; Eizaguirre et al. 2009*b*). Moreover, it was suggested that these condition-dependent signals reveal alleles of particularly high quality under currently prevalent parasites (Jäger et al. 2007), as was the case for a given MHC haplotype (No01.No12) which provided resistance against the common ecto-parasite *Gyrodactylus* sp. (Eizaguirre et al. 2009*b*). Selection based on olfactory cues and good gene indicators are thought to have complementary functions, driving local adaptation and maintenance of MHC polymorphism (Eizaguirre et al. 2009*b*). Nevertheless, MHC-based mate choice also strengthens reproductive isolation between populations (Eizaguirre et al. 2009*a*; Andreou et al. 2017).

**Box 4. Individual MHC allele diversity in three-spined stickleback**

The work reported in this thesis focuses on the exon II of the MHC region which encodes the highly variable peptide-binding beta chain region of the final MHC molecule (Lenz et al. 2009*a*). Previous work on MHC class II*β* loci in three-spined stickleback reported as many as 6 separate genomic regions (Sato et al. 1998), but this has been corrected downward to roughly 2-4 (Reusch and Langefors 2005). Owing to recent duplication events it is not possible to target these loci separately and several alleles per individual have to be differentiated (Reusch et al. 2004). The Single Strand Conformation Polymorphism method was used initially but exchanged for Reference Strand-mediated Conformation Analysis (RSCA) since the latter allowed tracking of specific alleles between cohorts and populations. Using RSCA in combination with plasmid libraries of MHC class II*β* alleles allows the identification of specific alleles and obtaining their nucleotide sequence. Moreover, it helped identify alleles that segregate together. Those MHC II*β* alleles with tight linkage we refer to as haplotypes (Lenz et al. 2009*a*)

**Thesis outline**

Despite much evidence for parasite-mediated natural and sexual selection on the evolution of host resistance in general, and on specific MHC alleles and individual MHC diversity in particular, little is known about how they interact with other concomitant selection pressures. This thesis addresses this knowledge gap from several angles (Figure 12): In Chapter 2, I (and coauthors) present evidence for trade-offs between immunity and lifetime reproductive success, which likely shape natural variation in immunocompetence between populations. We quantified the reproductive cost of mounting an adaptive immunity unnecessarily and at the same time, increased reproductive success of vaccinated individuals under selection by parasites. In Chapter 3, we investigate whether the addition of a predator has consequences on transgenerational coevolutionary dynamics. Specifically, we find that predation relaxes parasite-mediated selection and as such alters patterns of negative frequency-dependent selection on MHC haplotypes between generations. In Chapter 4, we ask whether parasite-mediated and MHC-based mate choice changes when a predator is added as

additional selection agent. The results suggest that mate choice for compatibility is weaker, whereas selection based on good genes is maintained. In Chapter 5, we ask whether temporal variation in parasite-mediated selection and coinfection cause differential selection on specific MHC haplotypes, similar to that observed across space. Using seasonal variation in the relationship between specific MHC alleles and parasite resistance, we identify both variable and stable fitness benefits of MHC alleles over time. In Chapter 6, we hypothesize that variation in MHC-based resistance across populations leads to distinct parasite-mediated sexual selection dynamics. By assembling a population with and without resistance-associated MHC alleles, we show mate choice for compatibility in the absence of good genes, but that females choose males with good genes when present. Finally, we place each finding in the broader context of coevolution in a complex world and discuss some of the remaining questions.

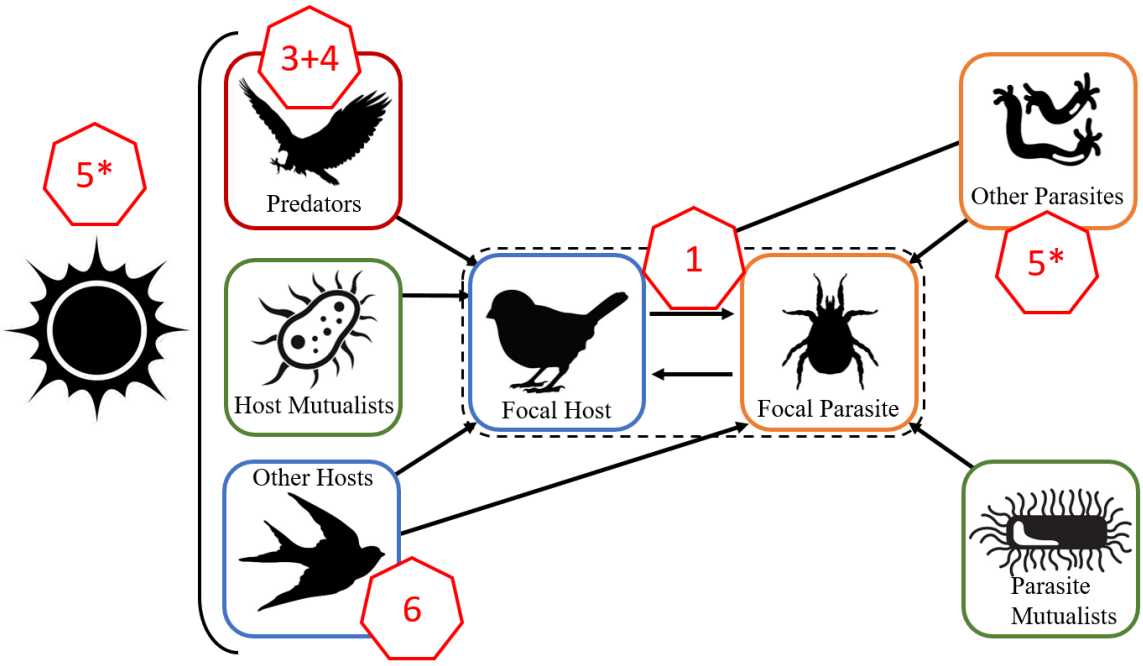


**Figure 12. Host-parasite coevolution occurs in a 'tangled bank'. The thesis plans to address several of these selection pressures.** Red heptagons with numbers show which concurrent selection pressure we examine and in which chapter. * indicated chapter that covers multiple aspects simultaneously.

# CHAPTER 2. COSTS AND BENEFITS OF ADAPTIVE IMMUNITY DEPEND ON THE STRENGTH OF PARASITE-MEDIATED SELECTION

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**Subject Areas:** host-parasite interaction

**Keywords:** adaptive immunity, resistance evolution, trade-offs, *Gasterosteus aculeatus*, cost of immunity, Darwinian fitness

## 2.1 Summary paragraph

As a result of recurrent parasite exposures, vertebrates have evolved several lines of defence including highly specific adaptive immunity (Klein and Figueroa 1986). Yet, not all populations are equally immuno-competent, likely as a result of different trade-offs emerging from distinct parasite pressures. Here, we experimentally tested the trade-off between immunocompetence and lifetime reproductive success using the three-spined stickleback fish as a model organism. We stimulated the antibody-mediated response of laboratory-bred fish by vaccinating them with an antigen homogenate (AG) derived from two common fish parasites. Vaccinated and control fish were then released into enclosures situated in a lake to be exposed to a diverse parasite fauna. Genetically similar replicated populations were released into macroparasite-free enclosures. We tracked individual lifetime reproductive success and found that parasite infection was costly, significantly reducing reproductive success (~39%) of PBS-injected fish between those populations exposed to parasites and those not experiencing parasite infection. Without parasites, vaccinated fish invested in immunocompetence at the expense of reproductive output (~23%). By contrast, in the parasite-rich environment, vaccination increased parasite-specific resistance and resulted in higher lifetime reproductive success compared to control fish (~10%). Our results provide an experimental quantification of the reproductive costs and benefits of an acquired immune response. As illustrated by our evolutionary model, such trade-offs explain the variation in immunocompetence observed across closely related populations and species exposed to different parasite loads.

## 2.2 Main text

Parasite infections are ubiquitous and reduce hosts' body condition, growth and reproductive investment, ultimately impacting a hosts' Darwinian fitness (Schmid-Hempel 2011). In response to this parasite-mediated selection, hosts have evolved responses to avoid infection (Behringer et al. 2006), tolerate it (Raberg et al. 2007) or ideally remain uninfected (Wegner et al. 2003*a*). Resistance comes at an evolutionary cost in the form of trade-offs with growth or condition emerging from limited resources (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Tschirren and Richner 2006). Yet, the most evolutionary relevant trade-offs are those involving reproduction (Sheldon and Verhulst 1996). But quantifying reproductive costs and benefits associated with resistance is difficult in nature due to multifarious selection pressures, the recurrence of infections and the difficulty to estimate lifetime reproductive fitness (Sheldon and Verhulst 1996; Bonneaud et al. 2003). Nevertheless, understanding the relationship between immunity and lifetime reproductive success is essential to understand the constrains leading to variation in immuno-competence across populations (Scharsack et al. 2007; Eizaguirre et al. 2012*a*), immune genes-mediated mate choice (Milinski 2006) and even parasite-mediated host speciation (Eizaguirre et al. 2009*a*; Feulner et al. 2015).

The adaptive immune system of vertebrates is central to the elimination of recurring parasite infection (Janeway et al. 2005). Tissue damage by helminths, for instance, elicits the secretion of Type 2 adaptive immune responses ($T_H2$) inducing cytokines (e.g. Interleukin-25) by the necrotic cells (Koyasu and Moro 2011). Dendritic cells, amongst others, then present parasite-derived antigens to naïve CD4+ T cells, activating $T_H2$ effector cell and follicular helper ($T_{FH}$) cell differentiation (Janeway et al. 2005). Cytokines and the $T_{FH}$ cells facilitate immunoglobulin E antibodies produced by B cells to bind to innate effector cells and hence, resulting in antigen-specific recognition and activation (Janeway et al. 2005; Koyasu and Moro 2011). During this process parasite-specific information is retained via T and B memory cells that persist after the initial infection (Janeway et al.

2005). Establishing such immunological memory is energetically costly (Martin II et al. 2003), but upon re-exposure it allows the adaptive immune system to orchestrate a rapid and highly parasite-specific immune response (Janeway et al. 2005).

Theory on the evolution of adaptive immunity postulates that individuals benefit from acquired immunity in the form of increased lifetime reproductive success in environments where recurrent parasite exposure is common. Reversely, without frequent parasite exposures, individuals would be predicted to carry the costs associated with mounting an initial immune response and building immunological memory. Despite these clear predictions, most studies thus far used proximal measures of reproductive success to assess cost/benefit trade-offs with immunity: Antigen-injected house sparrows (*Passer domesticus*) and pied flycatchers (*Ficedula hypoleuca*), for instance, reduce parental care and abandon their brood more readily, which in turn lowers offspring survival (Ilmonen et al. 2000; Bonneaud et al. 2003). Immunisation of male blackbirds (*Turdus merula*) with sheep red blood cells adversely affects the brightness of carotenoid-based beak colouration – a proxy of male reproductive success (Faivre et al. 2003). Since carotenoids play a crucial role in up-regulation of immune functions (Janeway et al. 2005), this outlines a trade-off in resource allocation between immune defences and reproductive success. Unequivocal experimental evidence for parasite-mediated trade-offs between immunity and lifetime reproductive success, however, is lacking.

The three-spined stickleback (*Gasterosteus aculeatus*) is host to a variety of parasites that impact its body condition, physiology, behaviour and lifetime reproductive success (Milinski and Bakker 1990; Eizaguirre et al. 2009*b*; Kalbe et al. 2009). Stickleback rapidly adapt to prevalent parasite species (Eizaguirre et al. 2012*b*; Weber et al. 2017), contributing to host local adaptation and population divergence (Eizaguirre et al. 2012*a*; Lenz et al. 2013; Feulner et al. 2015). Parasite-mediated selection is also important for female mate choice and the expression of carotenoid-based sexual ornaments in males (Eizaguirre et al. 2009*b*; Kalbe et al. 2009). Together this shows that parasite-mediated

selection plays a central role in determining the evolutionary trajectory of stickleback populations, and jawed vertebrates in general.

In order to experimentally induce immunological memory, we injected half of six laboratory-reared stickleback families with antigen homogenate (AG). Each antigen injection was synthesised from equal parts (1 µg) of two common parasites from the original stickleback population, i.e. *Diplostomum pseudospathaceum* and *Camallanus lacustris*, mixed with 4 µl Freund's complete adjuvant. The other half of each fish family was control-injected with a phosphate buffered saline solution (PBS; Figure 1A). Six parasite-free and parasite-exposed enclosures were stocked with 8 AG- and 8 PBS-injected fish from one family in equal sex ratio (Figure 1A, Kalbe et al. 2009). The parasite-rich enclosures are located in the lake of origin of the fish and allow for the passage of all major stickleback parasites, including *D. pseudospathaceum* and *C. lacustris,* and their intermediate hosts (Eizaguirre et al. 2009*b*; Kalbe et al. 2009). The parasite-free enclosures were located on land and supplemented with filtered water from the Schöhsee lake, to remove free-living parasites and all intermediate hosts which could carry macroparasites. On a weekly basis, nests were recovered from all enclosures, and individual lifetime reproductive success (LRS) was assessed via parenthood analysis based on 12 microsatellite markers for 24 randomly collected eggs from each clutch (Kalbe et al. 2009). After 9 weeks the fish were recovered from all populations, measured, dissected and screened for parasites, blind to the fish's identity (see supplementary material for detailed methods).

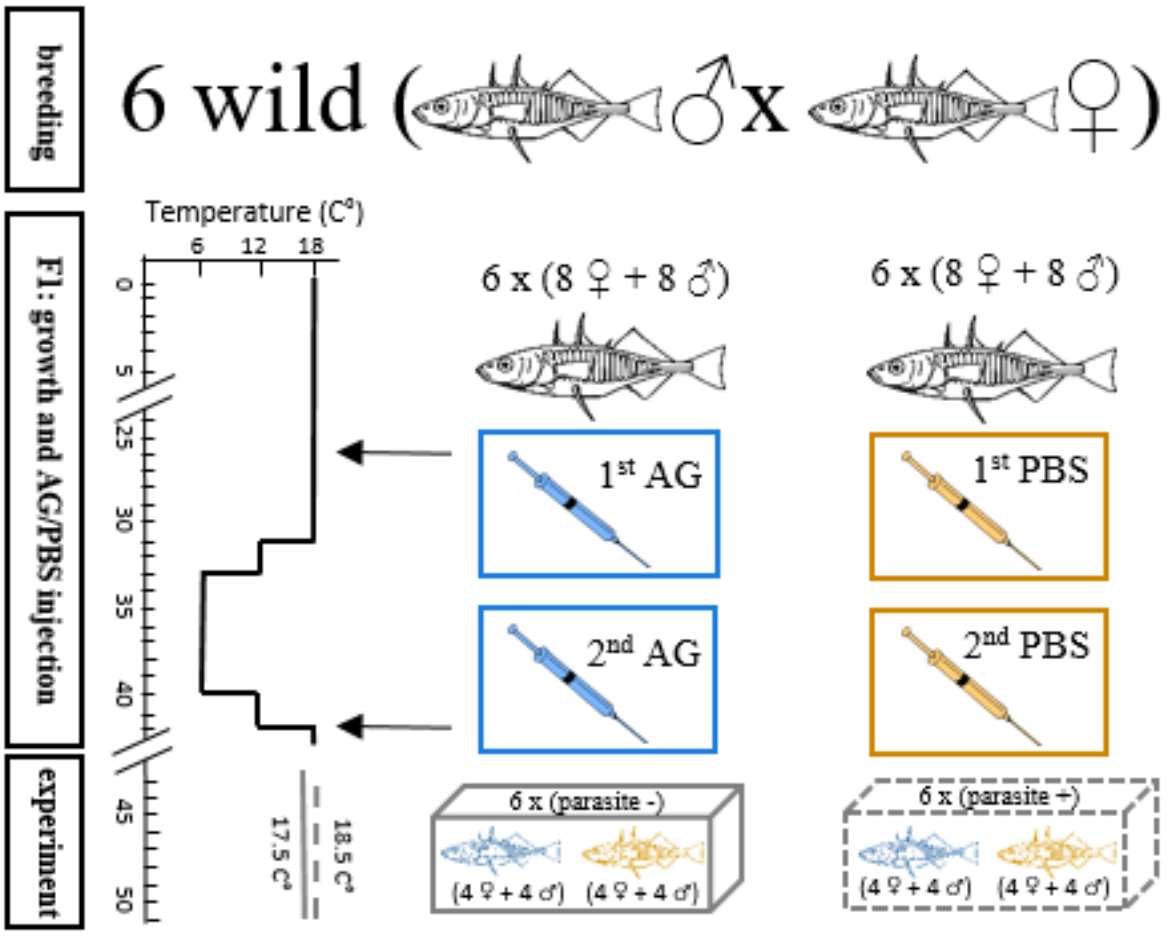


**Figure 1. (A) Six independent stickleback families were bred from wild fish from Großer Plöner See. All juveniles were reared under the same condition until week 26 when they were either injected with antigen (AG) or phosphate buffered saline (PBS) solution. Subsequently, all fish were brought into artificial autumn (12°C, 2 weeks), winter (6°C, 7 weeks), spring (12°C, 2 weeks) and back into summer (18°C) conditions to mimic the life cycle of sticklebacks. All fish were injected a second time before being released into a parasite-rich environment (N=6) in the lake of origin of the fish, or a parasite-free environment in artificial concrete ponds (N=6). Sixteen fish were released in equal sex ratio and equal proportion of AG-and PBS-injected from the same family per populations.**

Fish in the parasite exposed enclosures harboured on average 8.6 (±0.2 standard error) different parasite species from a total of 20 different parasite species identified. We genotyped a total of 3959 eggs from 139 clutches (parasite-exposed enclosure mean number of clutches ± standard error: 10.2 ±1.3; parasite-free enclosures: 12.2 ±2.3, students t-test: *d.f.*=7.9, t=0.76, p=0.469).

Following our main working hypothesis, we found that individual LRS was associated with a significant interaction between injection treatment and parasite environment ($F_{1,139}$=7.33, p=0.008, Figure 2A, Table 1): AG-injected individuals experienced reduced LRS (~23%) in parasite-free environments (reported as mean± standard error, PBS-injected: 49.1 ±6.1; AG-injected: 37.9 ±4.6; $F_{1,81}$=3.28, p=0.074), whereas LRS was ~10% higher for vaccinated fish in the parasite-exposed environments (PBS-injected: 29.5 ±3.0; AG-injected: 32.9 ±3.1; $F_{1,57}$=4.87, p=0.031). We further estimated the sole cost of parasitism to be a reduction of ~39% in LRS between PBS-injected fish from the different parasite exposure treatments (parasite-exposed: 29.5 ±3.0; parasite-free: 49.1 ±6.1). These results quantify the cost of mounting an adaptive immune response in the absence of re-occurring parasites and, importantly, demonstrate the evolutionary relevance of adaptive immunity upon re-exposure.

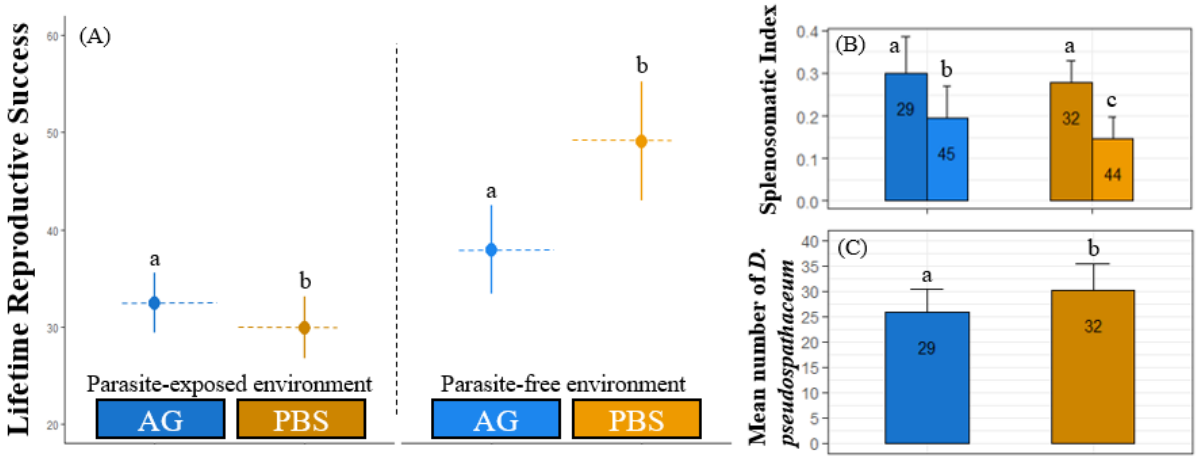


**Figure 2. (A) Mean individual lifetime reproductive success (±SE) differed between injection treatments (PBS-injection=yellow; AG-injection=blue) and parasite exposure (parasite-exposed=dark shade; parasite-free=light shade; two-way interaction: $F_{1,139}$=7.33, p=0.008, letters depicts main effects within respective parasite exposure treatment, Table 1). (B) The difference in splenosomatic index (+SE) between AG- and PBS-injected individuals (light blue and yellow, respectively) was larger in environments unexposed to parasites (two-way interaction: $F_{1,135}$=5.34, p=0.022; Supplementary Table 1). (C) The mean number of *D. pseudospathaceum* (+SE) parasites was approximately 15% lower in AG-injected (dark blue) than in PBS-injected individuals (dark yellow; $F_{1,44}$=4.82, p=0.034).**

**Table 1. Summary of linear mixed effect models with lifetime reproductive success as response variable, family and sex as random effects and injection treatment (AG/PBS), selection environment (lake/outside enclosure), body condition and parasite load (only within lake enclosure) as explanatory variables.** Models were backward selected using the *anova* function. Significant results are highlighted in bold, d.f. denotes degrees of freedom.

| data | explanatory variables | *d.f.* | f-value | p-value |
|---|---|---|---|---|
| Across selection environments | **Injection treatment** | 1,140 | 0.02 | 0.896 |
| | **Selection environment** | 1,133 | 3.74 | 0.055 |
| | **Body condition** | 1,113 | 1.22 | 0.272 |
| | **Injection treatment x selection environment** | 1,139 | 7.33 | **0.008** |
| | ***Pairwise comparison (Tukey)*** | | **Estimate (±SE)** | **p-value** |
| | **AG/Parasite + vs. PBS/Parasite +** | | 1.12 (0.61) | 0.265 |
| | **AG/Parasite + vs. AG/Parasite -** | | 0.03 (0.69) | 1 |
| | **AG/Parasite + vs. PBS/Parasite -** | | -0.99 (0.68) | 0.476 |
| | **PBS/Parasite + vs. AG/Parasite -** | | -1.09 (0.72) | 0.427 |
| | **PBS/Parasite + vs. PBS/Parasite-** | | -2.11 (0.71) | **0.018** |
| | **AG/Parasite - vs. PBS/Parasite -** | | -1.02 (0.51) | 0.191 |
| Parasite-exposed enclosures | **Injection treatment** | 1,57 | 4.87 | **0.031** |
| | **Parasite load** | 1,57 | 0.19 | 0.666 |
| | **Body condition*** | 1,57 | 0.32 | 0.572 |
| Parasite-free enclosures | **Injection treatment** | 1,81 | 3.28 | **0.074** |
| | **Body condition** | 1,81 | 1.57 | 0.214 |

*residuals of regression between body condition and parasite load are used

To ascertain that the differential LRS of the fish was due to the activation of adaptive immunity, we used both the splenosomatic index (SSI) and parasite load as further fitness proxies. Since dendritic cells in the marginal zone of the spleen present parasite-specific antigens to T cells, the spleen plays an important role in adaptive immunity and its weight correlates with LRS (Janeway et al. 2005; Kalbe et al. 2009). We found a significant interaction between injection treatment and selection environment on SSI ($F_{1,135}$=5.34, p=0.022, Figure 2C, Supplementary Table 1): fish exposed to parasites or injected with AG but unexposed to parasites had a higher SSI (all Tukey-test, p<0.05). The difference in SSI between AG- and PBS-injected fish under parasite exposure was, however, not significant as anticipated since PBS-injected fish also had to eventually mount a response against parasites in the lake enclosures (Tukey-test, p=ns, Supplementary Table 1). The high SSI of AG-

injected individuals in the parasite-unexposed enclosures reflects the costs of immunity in the absence of parasites.

As an additional control, we exposed the remaining AG- and PBS-injected fish to 100 cercaria of *D. pseudospathaceum* in a laboratory experiment (Supplementary Table 2). If efficient, our AG-treatment should increase parasite-specific resistance and hence, result in a lower infection with *D. pseudospathaceum.* This actively infecting digenean trematode impairs vision, reduces foraging efficiency and predator avoidance (Crowden and Broom 1980). We found that indeed AG-injection reduced *D. pseudospathaceum* infection by ~16% in the laboratory infections (PBS-injected: 8.5 ±0.7; AG-injected: 7.1 ±5.9; $F_{1,87}$=4.08, p=0.047, Figure 3A), matching the results from the field enclosure where AG-injected individuals had ~15% lower *D. pseudospathaceum* infection than their PBS-injected counterparts (PBS-injected: 30.3 ±4.2; AG-injected: 25.8 ±3.8; $F_{1,44}$=4.82, p=0.034, Figure 2B). When investigating the SSI fitness proxy in the laboratory, we also confirmed the same pattern as for fish from the enclosures where AG-injected individuals showed a higher splenosomatic index than PBS-injected fish ($F_{1,178}$=5.96, p=0.016, Figure 3B). These findings show that the antigens triggered memory cells formation and led to the development of parasite-specific immunity.

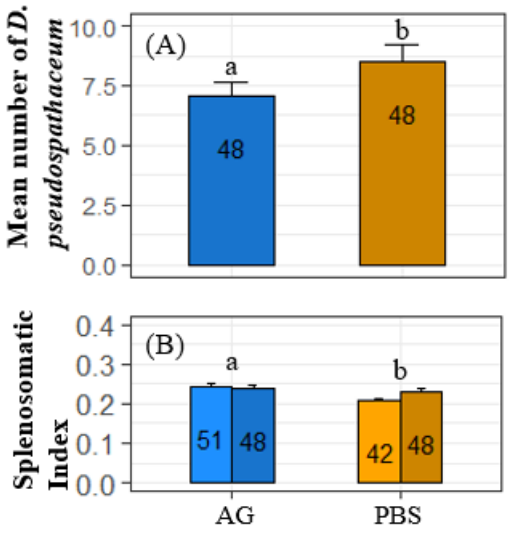


**Figure 3 (A) In the laboratory, individuals injected with AG (dark blue) had a significantly lower *D. pseudospathaceum* load than those injected with PBS (dark yellow; $F_{1,87}$=4.08, p=0.047).(B) Whether exposed to *D. pseudospathaceum* or not (light blue, light yellow respectively) individuals previously injected with AG (blue) had a higher splenosomatic index than those injected with PBS (yellow, $F_{1,178}$=5.96, p=0.016).**

Together, these results outline fundamental trade-offs between immunity and reproductive success controlled by the strength of parasite-mediated selection: The costs of infection was a ~39% reduction in LRS, which fits well in the range previously estimated in mite-infested swallows (18%, Møller 1993), blue tits infected with hen fleas (65%, Richner and Tripet 1999), or in sticklebacks infected by the cestode *Schistocephalus solidus* (23-91%, Heins et al. 1999). At the same time, investment into immunity is costly as demonstrated by a 23% decrease of LRS for vaccinated fish in the absence of parasites. Indeed cellular processes can be damaging to a host upon infection, as seen in three-spined stickleback populations where helminth development is halted by a fibrosis response that also reduces female fecundity by up to 89% in the wild (Weber et al. 2017).

Despite the costs of immunity and parasite infection, we found that fish that mounted a primary adaptive immune response from AG-injection, achieved a ~10% higher LRS compared to PBS-injected fish under repeated parasite exposure. Given the ubiquity of parasites, once adaptive immunity has evolved this quantified advantage should be sufficient to explain its fixation across

vertebrate taxa. We illustrate this evolutionary perspective by parameterizing a simple population-based adaptive model with values retrieved from our experiment (see supplementary material for model details). Assuming linear costs and benefits of infection and memory-mediated immunity, our model shows that the recorded parameters are sufficient to theoretically result in the rapid fixation of adaptive immunity (Figure 4).

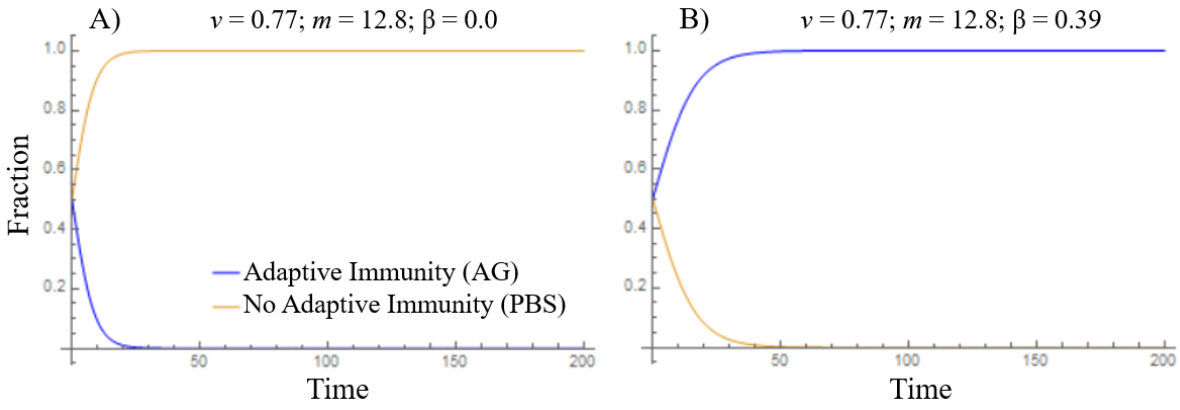


**Figure 4 (A) The costs associated with immunity (*v*) are selected against unless (B) parasite-mediated selection (β) imposes significant fitness costs, which immunity can partly overcome (*v*-*m*).** Time represents a unit of generation whereas fraction represents the frequency of individuals carrying the adaptive immunity trait or not. Model specifics are described in supplementary methods.

Together, the experimental data and the model show that trade-offs emerge from various strengths of parasite-mediated selection. The consequences of these trade-offs are optimised immune investments based on local likelihood of re-infection. For instance, riverine and lake stickleback populations face well described quantitative differences in the strength of local parasite-mediated selection, with re-infection being common within lake habitats (Kalbe and Kurtz 2006; Eizaguirre et al. 2011). This results in population-specific immune gene expression profiles (Lenz et al. 2013; Huang et al. 2016) and divergent selection on genes of the Major Histocompatibility Complex Class IIβ, a highly polymorphic region of the vertebrate genome at the center of adaptive immunity (Eizaguirre et al. 2012*a*).

In summary, we show that the strength of parasite-mediated selection shapes the trade-offs between the costs associated with parasite infection, the costs of mounting a specific immune response and the benefits of adaptive immunity. Our data explains the different patterns of resistance across populations of jawed vertebrates.

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**Author contribution.** MK, MM and CE conceived and performed the experiment. MK and CE collected the data. DS performed the parenthood analyses and analysed the data with CE and MK. DS and CE drafted the manuscript. All authors contributed to the final version of the manuscript.

# CHAPTER 3. PREDATION RELAXES PARASITE-MEDIATED SELECTION AND ALTERS THE EVOLUTION OF MHC-BASED RESISTANCE TO PARASITES

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**Subject Areas:** host-parasite interaction

**Keywords:** predation, Major Histocompatibility complex, host-parasite interactions, *Gasterosteus aculeatus*, negative frequency-dependent selection

**Abstract**

Predators and parasites are major selective agents in nature, yet their interplay remain largely unexplored. Here, we studied experimentally whether predation alters the evolution of parasite resistance, focusing on a well-characterized genetic basis of immunity in jawed vertebrates, the genes of the major histocompatibility complex (MHC). In a field enclosure experiment, wild-caught three-spined stickleback fish were exposed to either only their natural parasite fauna or to both parasites and a predatory pike simultaneously. We show that without predation, the mean individual parasite load was positively associated with MHC haplotype frequency, while the mean individual lifetime reproductive success negatively correlated with MHC haplotype frequency in the populations. We also found adaptive shifts of rare resistance MHC haplotypes across generations, following predictions from the Red Queen hypothesis. Interestingly, no such relationship between MHC haplotype frequency and fitness was observed under predation. Instead, we found that, under simultaneous predation and parasite pressures, the frequency of the most common MHC haplotype increased across generations. This is because predation reduced overall competition, and relaxed parasite-mediated selection allowing those fish carrying a common MHC haplotype to establish territories and gain mating opportunities. Overall, by altering population dynamics, predation impacts the evolution of MHC-mediated resistance to parasites.

## Introduction

Predator-prey and host-parasite interactions are characterised by successive adaptations and counter-adaptations resulting in two main evolutionary dynamics: recurrent selective sweeps (i.e. arms race) and negative frequency-dependent selection (i.e. Red Queen dynamics, Van Valen 1973; Lively 2010*a*). While both mechanisms assume a selective advantage of rare genotypes, they differ in the way genetic variants spread within the population. Recurrent selective sweeps mainly rely on the successive and rapid fixation of novel beneficial variants emerging from *de-novo* mutations or migration (e.g. Buckling and Rainey 2002; Cook et al. 2012). Negative-frequency-dependent selection, on the other hand, relies on standing genetic variation and the oscillation of rare genotypes present in the population (e.g. Dybdahl and Lively 1998; Decaestecker et al. 2007; Hiltunen and Becks 2014). While in isolation we begin to understand the dynamics underlying predator-prey and host-parasite coevolution, how predation- and parasite-mediated selections interact to shape prey/host trait evolution is largely unexplored.

Predators select for individuals with specific morphological traits such as spines in fish (Hoogland et al. 1956) or horns in large-bodied African antelopes (Packer 1983). They also select for anti-predator behaviour such as cooperative predator inspection (Milinski 1987) or chemical predator recognition (Chivers and Smith 1995). The sole presence of a predator can also harm prey by lowering foraging success (Milinski and Heller 1978; Milinski 1986; Higginson et al. 2012). As a consequence, by inducing stress, predation can increase parasite transmission, infection success and virulence (Navarro et al. 2004; Best 2018). Combined with the fact that parasites also reduce foraging efficiency, host condition and lessen anti-predator behaviour (Crowden and Broom 1980; Milinski 1985, 1990; Milinski and Bakker 1990), it is not surprising that predation is hypothesized to act against the weakest, often parasitized, individuals within a population (e.g. Eutermoser 1961; Lafferty and Morris 1996). In this context, the evolution of parasite resistance can be viewed as a functional

trait, for host and prey alike, that reduces infection and, hence, should reduce infection-dependent predation costs (Best 2018).

However, severe predation pressure can also dramatically reduce host densities. Population size plays a vital role in host-parasite dynamics (Papkou et al. 2016), impacting transmission and virulence of parasites (Lively et al. 1995; Eizaguirre et al. 2009*b*). As host density declines so does the diversity in host genotypes, which has negative effects on host infection rate (Lively 2010*c*). Such indirect density-mediated predation effects can disrupt coevolutionary dynamics between host and parasite, thereby slowing down the evolution of host resistance (Frickel et al. 2017).

As part of the adaptive immune system of jawed vertebrates, the genes of the major histocompatibility complex (MHC) class II*β* are the best characterised genetic basis of parasite resistance (Janeway et al. 2005). This highly polymorphic genomic region encodes a suite of structurally related yet distinct molecules, which present parasite-derived antigens to T cells to mount parasite-specific immune responses (Janeway et al. 2005). Negative frequency-dependent selection (NFDS), heterozygote advantage and habitat heterogeneity jointly contribute to the maintenance of the exceptional MHC polymorphism (Eizaguirre and Lenz 2010; Spurgin and Richardson 2010). Specifically, elements of NFDS, as a mechanism of the evolution of parasite resistance, have been described with evidence of i) rare MHC genotypes to provide increased resistance against parasites (Phillips et al. 2018) as well as ii) the demonstration of adaptive frequency shifts of resistance MHC alleles across generations (Eizaguirre et al. 2012*b*). These findings, however, remains independent of other selection pressures, including predation, and it is unclear how they interact with each other. This knowledge gap is likely bigger than anticipated, since the evolution of MHC-based resistance to parasites is also linked to sexual selection and mate choice in particular, both of which are also known to be altered by predation pressures (e.g. Reznick et al. 1990; Milinski 2006; Johnson and Candolin 2017).

The three-spined stickleback (*Gasterosteus aculeatus*) is an ideal model species to study how predator- and parasite-mediated selections affect the evolution and maintenance of MHC diversity. Predation pressure on sticklebacks negatively impacts foraging behaviour and condition (Milinski and Heller 1978; Milinski 1985, 1986). Parasites, on the other hand, increase predation risk because they lower host condition by modifying foraging efficiency (Milinski 1984, 1986; Anaya-Rojas et al. 2016) as well as reduce the efficiency of anti-predator behaviours (Milinski 1985). Furthermore, MHC genes are well characterised for this species (Lenz et al. 2009*a*) and both specific MHC alleles and individual MHC diversity determine parasite resistance (Wegner et al. 2003*b*; Eizaguirre et al. 2012*b*) and female mate choice (Milinski et al. 2005; Milinski 2015). Since resistance MHC alleles are associated with increased lifetime reproductive success (Eizaguirre et al. 2009*b*; Kalbe et al. 2009), they increase in frequency in the next generation (Eizaguirre et al. 2012*b*; Milinski 2015), consistent with NFDS. But does predation alter those well-characterized dynamics?

Using field enclosures, we exposed wild-caught three-spined sticklebacks to either only their local parasite fauna or to both, parasites and a native predatory pike simultaneously. Sticklebacks were able to hide from the pike, feed and reproduce freely in their large enclosures located in their natal lake (Kalbe et al. 2009). We monitored their survival and collected fertilized eggs from the sticklebacks' nests on a weekly basis to determine individual lifetime reproductive success via a molecular parenthood analysis of the eggs. At the end of the experiment, fish were dissected to determine the relationship between their parasite load, lifetime reproductive success and the presence of specific MHC haplotypes. We also determined the frequency change of MHC haplotypes across generations to assess how predation alters the evolution of MHC-based resistance. We hypothesized that predation removes individuals with high parasite load and, consequently, removes susceptible MHC haplotypes from the reproductive pool. This should result in predation favouring MHC resistance haplotypes, which are likely to be rare, hence, accelerating negative-frequency-dependent selection. At the same time, density-mediated effects of predation might impact these dynamics.

**Materials and Methods**

*FISH COLLECTION AND EXPERIMENTAL DESIGN*. In April 2011 and 2012, three-spined sticklebacks were caught from the lake Großer Plöner See (54°9'21.16'N, 10°25'50.14'E, Germany). After capture, a small spine clip was taken from all fish for DNA extraction and later, microsatellite-based individual identification. Fish were measured, weighed and randomly distributed across six groups, each containing twelve males and twelve females, making it a total of 72 fish per treatment for each year. Each group was then released into one of six enclosures (3x3m stainless steel fence, height of 1m, 0.4-0.6m above the water surface, 0.5cm mesh size, Eizaguirre et al. 2009; Kalbe et al. 2009) within their lake of origin. The mesh of the enclosures allows for the passage of prey, parasites and their intermediate hosts. The enclosures also contained structural elements (stones, plants, wooden debris, etc.) used by male sticklebacks for nest-building and by both sexes for shelter (Eizaguirre et al. 2009*b*; Kalbe et al. 2009). For the purpose of this experiment, in each year, three of the six enclosures (2011: Enclosure 1, 3, and 5; 2012: Enclosure 2, 4, and 6) were stocked with a Northern Pike (*Esox lucius*, 30-40cm), a natural predator of three-spined sticklebacks. Each enclosure was protected against avian predation by a net. Enclosures were inspected weekly to record survival rate and if dead fish were observed, they were collected.

*FISH PARASITE LOAD & CONDITION INDICES*. At the end of the experiment, all surviving fish were recaptured, dissected and screened for ecto- and endo-parasites (Kalbe et al. 2009). Parasite numbers and diversity were combined into an individual parasite index ($I_{PI}$, Kalbe et al. 2002). At this stage, fish were also measured, weighed and a fin clip was taken for genetic identification. Weights and lengths before and after the experiments were used to calculate the initial and final body conditions: $(\text{weight}/\text{length})^b \times 100$ with *b* being set at 3.00 (Frischknecht 1993).

*MICROSATELLITES AND MAJOR HISTOCOMPATIBILITY COMPLEX GENOTYPING*. DNA extractions, from dorsal spines (before the experiment) and fins (after the experiment), were performed using DNeasy 96 Blood & Tissue Kit (Qiagen) following the manufacturers' protocol. All

fish were genotyped for nine microsatellites combined in two different multiplexed PCRs (Kalbe et al. 2009). The MHC class II*β* genotypes of all fish were determined using reference-strand-mediated conformation analysis (RSCA) optimized for three-spined sticklebacks (Lenz et al. 2009*a*). We targeted the exon II of the MHC class II genes, which encodes the highly variable peptide-binding beta chain region of the final molecule. Notably, the MHC II*β* genes in stickleback are duplicated and frequently occur in tightly linked alleles, which we refer to as haplotypes (Lenz et al. 2009*a*).

*EGG COLLECTION, LIFETIME REPRODUCTIVE SUCCESS AND INFERRED OFFSPRING MHC GENOTYPES.* On a weekly basis, all enclosures were inspected for nests and all egg clutches collected (Kalbe et al. 2009). Twenty-four randomly collected eggs of each clutch were used for DNA extraction and later parenthood analysis. Extraction took place on a Freedom evo robot (Tecan) using Invisorb Tissue HTS 96 kit/R (Stratec). A total of 277 nests were collected and 14,742 eggs were genotyped for parenthood analysis based on nine microsatellites using the software PAPA (Eizaguirre et al. 2009*b*). Subsequently, individual lifetime reproductive success (LRS) was determined for all fish and nest ownership was established for all males (Eizaguirre et al. 2009*b*). We then combined the information gained from the parenthood analysis and parental MHC genotypes to calculate a probabilistic abundance of each of the codominant MHC haplotypes in the next generation (Janeway et al. 2005).

*DATA ANALYSES.* All statistical analyses were conducted with R version 3.3.1 (R Core Team 2016; packages include: '*vegan*', '*lmerTest*', '*lme4*'). Model residuals were tested for normality and homoscedasticity of variance. Data were transformed if required to meet test assumptions. All models were backward-selected using the *anova* function.

*EXPERIMENTAL SET-UP: MHC DIVERSITY AND BODY CONDITION.* We confirmed a balanced experimental design, showing no differences in individual MHC allele number across enclosures within year using a Kruskal-Wallis rank test (2011: $x^2_5$=5.39, p=0.369; 2012: $x^2_5$=8.79, p=0.118) and

a Wilcoxon rank-sum tests for the difference between years (W=10177, p=0.939) as well as between treatments across years (Supplementary Table S1; 2011: W=2408, p=0.600; 2012: W=2439, p=0.479). Similarly, there was no difference between MHC haplotype pools between treatments as revealed by an ANOSIM (1000 permutations) for each year (Supplementary Table S1; 2011: Global R= -0.010, p=0.930; 2012: Global R= -0.007, p=0.821). Lastly, we show with a linear mixed effect model with treatment as a fixed variable and enclosure and year as random factors that there was no bias with respect to fish initial body condition across enclosures at the start of the experiment ($F_{1,10}$=0.154, p=0.703).

*SURVIVAL RATE, LIFETIME REPRODUCTIVE SUCCESS AND PARASITE LOAD.* A generalized mixed effect model was used to determine the likelihood of survival (i.e. binomial variable) using treatment, sex, initial body condition and MHC haplotype zygosity (homozygote vs heterozygote) as well all two-way interactions as fixed factors with year and enclosure as random factors. Secondly, we estimated the effect of treatment, sex, MHC haplotype zygosity, initial body condition and parasite load (expressed as residuals of the regression between parasite load and initial body condition) on individual lifetime reproductive success (LRS, square root-transformed) using a mixed effect model also with year and enclosure as random factors. Focusing on male fish, we tested the effect of predation treatment, MHC haplotype zygosity, initial body condition and parasite load (expressed as residuals of regression between parasite load and initial body condition) on the number of nest owned (Poisson distribution) with a similar generalised mixed effect model also using year and enclosure as random factors. Furthermore, the same model structures were used to identify the determinant of final body condition (log-transformed) and parasite load ($I_{PI}$, log-transformed), but removing them from the explanatory variables in their respective models. Lastly, the parasite communities were compared between treatments using a PERMANOVA with year set as a block factor.

*MHC HAPLOTYPE COMPOSITION OF SURVIVORS AND OFFSPRING*. MHC haplotype compositions of the surviving fish were compared between treatments with a PERMANOVA for each

year separately. Secondly, to test for differences between MHC haplotype composition within the offspring generation (either emerging from control or predation treatments), we compared their estimated MHC haplotype composition determined from parenthood analysis between treatments with a PERMANOVA with year set as a block factor. Following the PERMANOVA, a similarity percentage analysis (SIMPER, set to 1000 permutations) was performed to identify which MHC haplotypes contributed most to the observed difference between offspring groups (Eizaguirre et al. 2012*b*).

**Results**

*SURVIVAL*. From a starting total of 72 individuals, 69 (♀=34, ♂=35) and 65 (♀=35, ♂=30) individuals were recovered from the control enclosures in 2011 and 2012, respectively, and after 39 and 54 days in the enclosures. The second experimental block lasted longer than the first to allow for comparable predation rates between years. Twenty-seven individuals (♀=15, ♂=12) in 2011 and 24 (♀=17, ♂=7) in 2012 survived the predation treatment. As expected, predation resulted in increased mortality compared to control ($F_{1,195}$=10.40, p<0.001; Supplementary Table 2a) and interestingly more males died than females ($F_{1,195}$=10.69, p=0.001), resulting in a skewed sex ratio in the predation treatment. Interestingly, in one enclosure in 2011 predation only removed three individuals over the course of the experiment.

*PARASITE LOAD AND COMMUNITY.* Individual parasite load was higher in the control than in the predation treatment at the end of the experiment (Figure 1A; Supplementary Table 2b; $F_{1,141}$=7.67, p=0.006), with notable outliers in the enclosure with little predation. Furthermore, we found a treatment by initial body condition interaction (Figure 1B; $F_{1,171}$=5.88, p=0.016), showing that low condition fish with higher parasite load had been removed from the populations by the predator.

Noteworthy, the parasite community composition harboured by the surviving fish did not differ significantly between treatments at the end of the experiment (PERMANOVA: $F_{1,181}$=1.48, p=0.172).

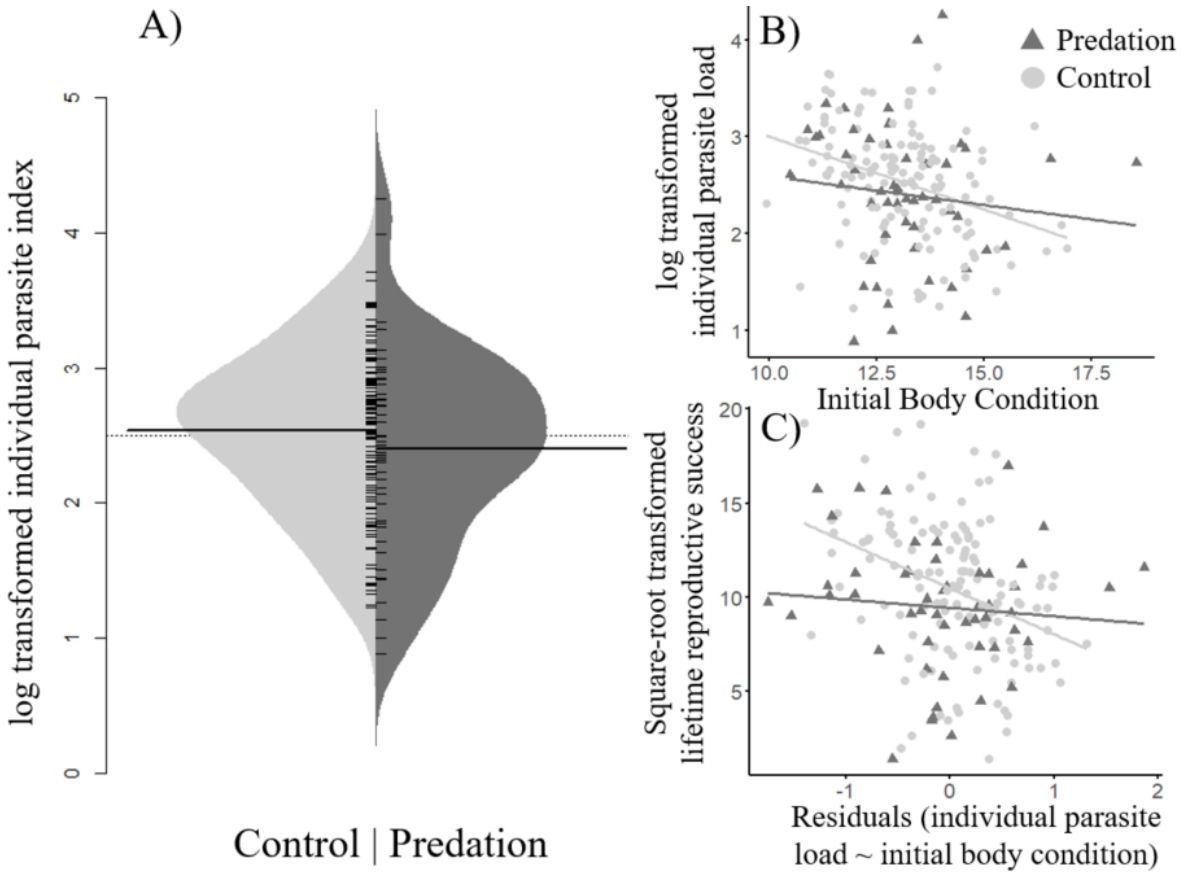


**Figure 1. A) Individual parasite load was significantly lower in the predation treatment (dark grey) than in the control group (light grey; black line denotes means). B) Parasite load ($I_{PI}$) was more strongly negatively correlated to initial body condition under control (light grey circles) condition than under predation (dark grey triangles). C) Similarly, lifetime reproductive success was more significantly negatively correlated with $I_{PI}$ in the control than the predation treatment.**

*FINAL BODY CONDITION*. The final body condition of the surviving fish did not significantly differ between treatment groups (Supplementary Table 2c; $F_{1,9}$=0.86, p=0.378), but was higher in males than females ($F_{1,170}$=66.20, p<0.001), and MHC heterozygous individuals compared to homozygotes ($F_{1,169}$=4.45, p=0.036). We also found that final body condition was positively associated with initial body condition ($F_{1,176}$=60.12, p<0.001).

*LIFETIME REPRODUCTIVE SUCCESS*. Individual LRS was positively associated with initial body condition (Supplementary Table 2d; $F_{1,116}$=7.31, p=0.008) and negatively with parasite load overall (Figure 1C; $F_{1,46}$=9.31, p=0.004), independently of the treatment the fish were in ($F_{1,5}$=2.93, p=0.152), demonstrating the general cost of parasitism.

*NEST OWNERSHIP*. The number of nests maintained by each male during the course of the experiment was not significantly different between treatments nor between homo- and heterozygous individuals, but was weakly positive related to initial body condition ($F_{1,82}$=4.50, p=0.072) and was strongly negatively associated with parasite load ($F_{1,82}$=10.09, p=0.001).

*MHC HAPLOTYPE COMPOSITION OF SURVIVORS AND OFFSPRING*. The pools of MHC haplotypes of the surviving fish did not differ significantly between treatment and control populations in either year (PERMANOVA, 2011: $F_{1,93}$=-0.17, p=0.979; 2012: $F_{1,86}$=0.47, p=0.769), but varied between treatments in the offspring generation (Supplementary Figure 1; PERMANOVA, $F_{1,1440}$=92.24, p<0.001). A subsequent SIMPER analysis highlighted that haplotype No13.No18 (alleles No13 and No18, NCBI accession numbers AF395711 and AY687846, respectively) was consistently more common in the offspring of the predation populations (SIMPER, p<0.001). Interestingly, we showed that 8 haplotypes had significantly different abundances between treatments and all of them, except No07.No31, revealed to be more common under predation than under control conditions, suggesting different MHC frequency dynamics between predation and control populations (haplotypes No18.No13, So05.So11. SCX03, No25.No27, No42.No45, No36.No54, No55, No15.No62; see Supplementary Table 3).

*PARASITE-MEDIATED FREQUENCY-DEPENDENT SELECTION ON MHC HAPLOTYPES*. Since the parental MHC haplotype pool was comparable between treatments, the difference in offspring MHC composition must originate from differential mating dynamics in the parental populations between treatment groups. To test the *posteriori* hypothesis, we first calculated change (Δ) in MHC

haplotype frequency from one generation to the next as Δ = offspring MHC haplotype frequency – parental MHC haplotype frequency (Koskella and Lively 2009). This change in MHC haplotype frequency was then used as a response variable with treatment and parental MHC haplotype frequency as explanatory variables and enclosure and year set as random effects. The results indicate an interaction between treatment and parental MHC haplotype frequency (Figure 2A; $F_{1,142}$=3.84, p=0.052): In the control treatment, as parental frequency of MHC haplotypes increased, their frequency was more likely to be lower in the next generation ($F_{1,69}$=4.34, p=0.041), showing that frequencies of initially common MHC haplotypes decreased in the next generation. Under predation pressure, MHC haplotype frequency change was not correlated with parental MHC haplotype frequency ($F_{1,73}$=0.57, p=0.451), suggesting that predation alters MHC frequency dynamics compared to parasite-mediated selection alone.

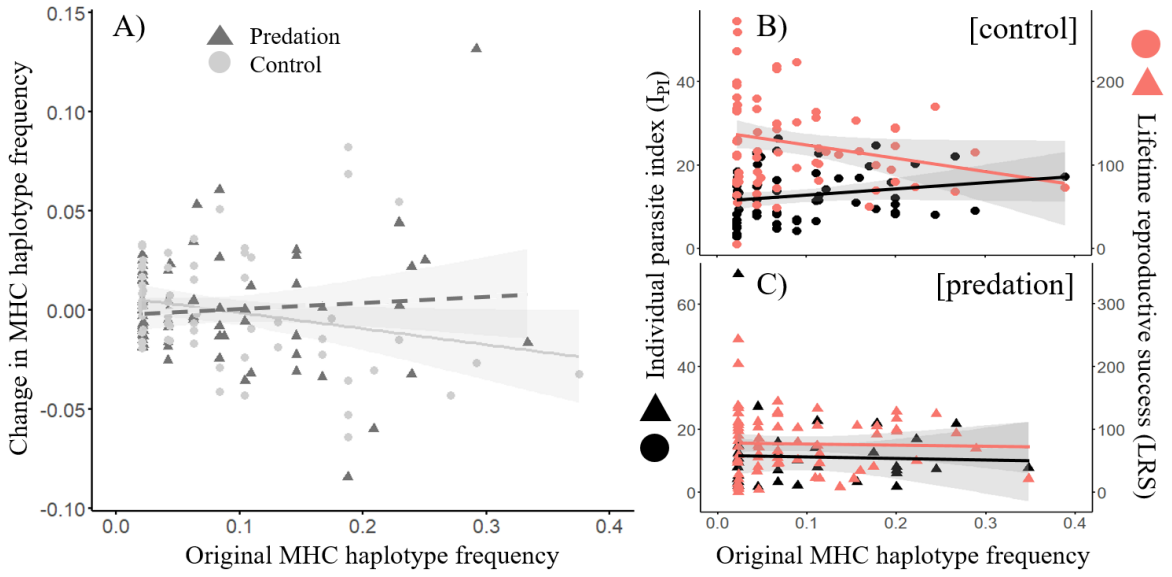


**Figure 2. A) Under sole parasite-mediated selection common MHC haplotypes decreased in frequency in the next generation (light grey circles), but no such correlation was detected under predation (dark grey triangles). B) In the control, parental MHC haplotypes frequency was positively correlated with parasite load (black circles) and negatively with lifetime reproductive success (pink circles), but C) under predation none of those correlations were significant.**

Based on these results we hypothesised that on average fitness advantages decrease with increasing MHC haplotype frequency. We therefore correlated mean parasite load as well as reproductive success for each haplotype with the mean parental MHC haplotype frequencies derived from replicated populations of each treatment. We found mean parasite load correlated positively with MHC haplotype frequencies across control enclosures (Figure 2B; Spearman's $\rho$=0.33, p=0.006), showing that on average individuals with common MHC haplotypes were more infected than individuals carrying rarer haplotypes following the pattern expected under NFDS. Such a pattern was not detected under predation (Figure 2C; Spearman's $\rho$=0.15, p=0.403). Likely as a consequence of lower mean parasite load in fish carrying rare MHC haplotypes, mean individual LRS was negatively associated with increasing MHC haplotype frequency in the control enclosures (Figure 2B; Spearman's $\rho$=-0.25, p=0.043), but showed no relationship with MHC frequencies in the predation treatment (Figure 2C; Spearman's $\rho$=-0.017, p=0.888).

*SPECIFIC MHC HAPLOTYPE FITNESS BENEFITS*. Given the selective advantage of specific MHC haplotypes, we re-conducted previous analyses on survival, parasite load, final body condition and LRS adding the most differentially selected MHC Haplotype No13.No18 as an explanatory variable (Table 1). The other haplotypes identified with the SIMPER were too rare for robust statistics.

**Table 1. Statistical summary table showing the effects of treatment, the presence and absence of MHC haplotype No13.No18 and tested explanatory variables on a) survival, b) individual parasite load, c) final body condition, d) lifetime reproductive success and e) nest ownership.** All models were backward selected using the *anova* function in R. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| **a) Survival** | ***d.f.*** | **F-value** | **p-value** |
|---|---|---|---|
| **Sex** | 1,285 | 7.13 | **0.001** |
| **Treatment** | 1,285 | 13.42 | **<0.001** |
| **Initial body condition** | 1,285 | 0.08 | 0.850 |
| **Zygosity** | 1,285 | 0.68 | 0.413 |
| **Haplotype No13.No18** | 1,285 | 1.73 | 0.180 |

**b) Individual Parasite Load ($I_{PI}$)**

| | | | |
|---|---|---|---|
| **Sex** | 1,165 | 0.35 | 0.556 |
| **Treatment** | 1,129 | 11.16 | **0.001** |
| **Initial body condition** | 1,167 | 0.54 | 0.462 |
| **Zygosity** | 1,166 | 1.60 | 0.208 |
| **Haplotype No13.No18** | 1,166 | 1.81 | 0.180 |
| **Sex * Haplotype No13.No18** | 1,166 | 11.08 | **0.001** |
| **Treatment * Initial Body Condition** | 1,167 | 8.86 | **0.003** |
| **Treatment * Haplotype No13.No18** | 1,166 | 5.82 | **0.017** |

**c) Final Body Condition**

| | | | |
|---|---|---|---|
| **Sex** | 1,168 | 65.57 | **<0.001** |
| **Treatment** | 1,9 | 0.66 | 0.437 |
| **Initial body condition** | 1,173 | 55.31 | **<0.001** |
| **Zygosity** | 1,168 | 9.45 | **0.002** |
| **Haplotype No13.No18** | 1,168 | 4.89 | **0.028** |
| **Parasite load corrected for initial body condition** | 1,149 | 3.20 | 0.076 |
| **Haplotype No13.No18 * Zygosity** | 1,170 | 6.29 | **0.013** |

**d) Lifetime reproductive success (LRS)**

| | | | |
|---|---|---|---|
| **Sex** | 1,164 | 0.13 | 0.718 |
| **Treatment** | 1,3 | 2.84 | 0.184 |
| **Initial body condition** | 1,79 | 8.09 | 0.006 |
| **Zygosity** | 1,174 | 0.02 | 0.893 |
| **Haplotype No13.No18** | 1,174 | 0.10 | 0.754 |
| **Parasite load corrected for initial body condition** | 1,25 | 10.57 | **0.003** |
| **Treatment * Parasite load corrected for initial body condition** | 1,26 | 4.21 | **0.050** |
| **Treatment * Haplotype No13.No18** | 1,174 | 2.95 | 0.088 |

**e) Nest ownership**

| | | | |
|---|---|---|---|
| **Treatment** | 1,82 | 1.30 | 0.285 |
| **Initial body condition** | 1,82 | 3.96 | 0.076 |
| **Zygosity** | 1,82 | 0.28 | 0.596 |
| **Haplotype No13.No18** | 1,82 | 2.43 | 0.647 |
| **Parasite load corrected for initial body condition** | 1,82 | 8.61 | **<0.003** |

Individuals with haplotype No13.No18 did not show differential survival between treatments (Table 1a) nor did the number of nests maintained differ amongst males with and without haplotype No13.No18 (Table 1e). We found, however, an interaction between the presence of this haplotype

and treatment on parasite load, indicating a context-dependent resistance effect of this MHC haplotype (Table 1b; Figure 3A; haplotype * treatment: $F_{1,166}$=5.82, p=0.017): Individuals with No13.No18 had higher parasite load under control than predation conditions. In addition, we found that males with this haplotype were more heavily infected than females (haplotype * sex: $F_{1,166}$=11.08, p=0.001). Heterozygote individuals for haplotype No13.No18 had a higher final body condition compared to homozygous individuals for this haplotype (Table 1c; haplotype * zygosity: $F_{1,170}$=6.29, p=0.013) independently of the treatment. Interestingly, individuals with No13.No18 in the control populations also tended to experience reduced lifetime reproductive success, whereas under predation they tended to experience higher LRS (Table 1d; Figure 3B; haplotype * treatment: $F_{1,174}$=3.33, p=0.088). Lastly, we found an interaction between parasite load and treatment on LRS, with a stronger negative relationship under control conditions than under predation (Table 1d; Figure 1C; treatment * $I_{PI}$: $F_{1,26}$=4.21, p=0.050), suggesting relaxed parasite-mediated selection under predation.

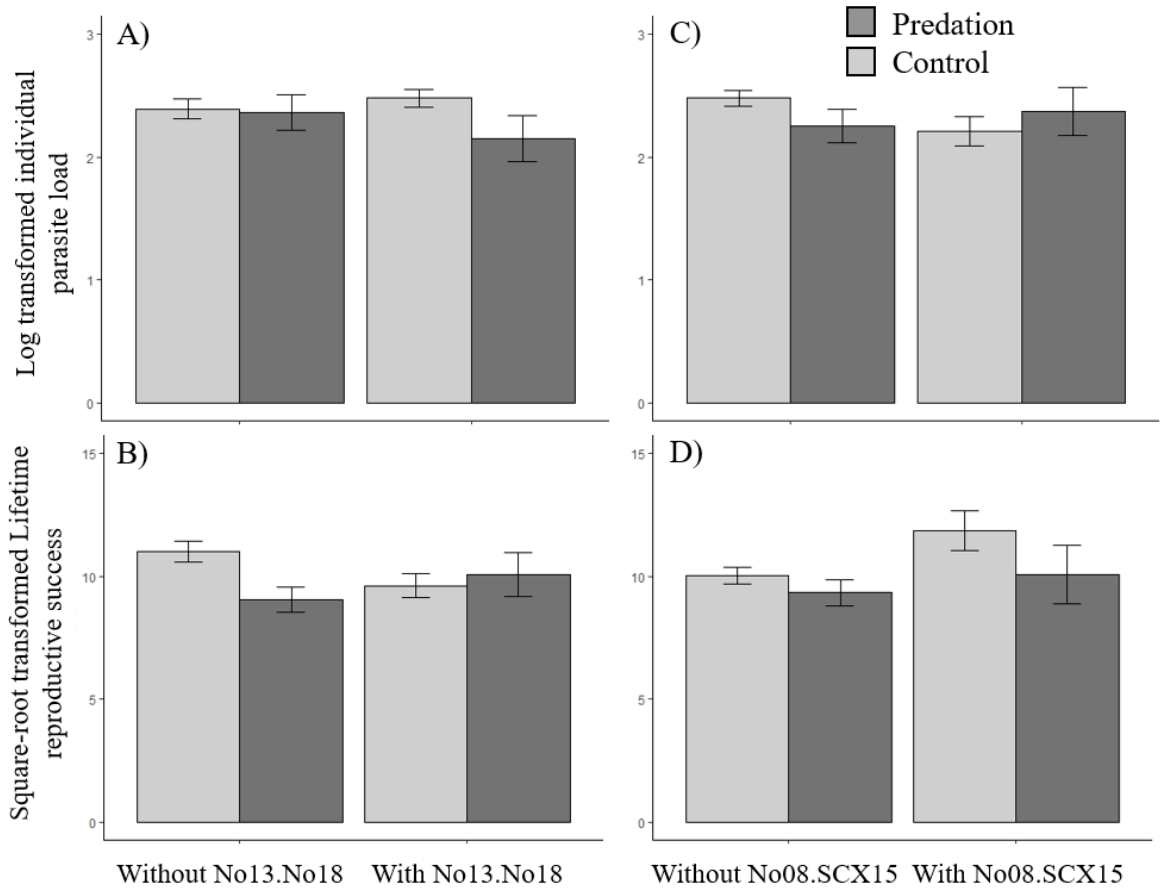


**Figure 3. A) Individuals with MHC haplotype No13.No18 had a lower parasite load ($I_{PI}$; shown as mean ± standard error) under predation (dark grey) compared to control (light grey) fish with this haplotype. B) This translated into reduced lifetime reproductive success of individuals without haplotype No13.No18 under predation. C) Individuals with haplotype No08.SCX15 had reduced $I_{PI}$ and D) increased LRS.**

*RARITY VS SPECIFIC MHC HAPLOTYPE EFFECTS.* To test whether NFDS was the result of MHC haplotype rarity alone or linked to the nature of the haplotype instead, we used a student t-test to compare the mean proportion of eggs sired by individuals with rare MHC haplotypes (<5% in the parental population) between treatments and found no differences (control: mean 9.1% ±0.7 standard error=SE; predation: mean 12.6% ±3.1SE; d.f. = 35, t=1.10, p=0.277). This suggests that MHC rarity alone is not sufficient for fitness advantages, and instead the specific resistance advantage of certain MHC haplotypes was the driver of the dynamics.

*MALE COMPETITION FOR TERRITORIES AND MATING OPPORTUNITIES*. Lastly, the reduction in male density due to mortality may lead to a reduction in competition for territories and nesting opportunities, contributing to differential reproductive success of haplotypes between treatments, particularly of individuals with rare or common MHC. We first compared the mean number of nests per enclosure between treatments using a student t-test and second, used a PERMANOVA comparing the MHC haplotype composition of frequent and rare nest owners between treatments with year as a block factor. The mean number of nests was tentatively higher in the control then predation treatment (control: mean 25 ±1.9SE; predation: mean 17.5 ±3.1SE; d.f. = 8.4, t=2.05, p=0.073), suggesting more competition at higher density in the control. Second, we found a significant difference as to which males acquired mating opportunities between treatments (PERMANOVA, $F_{3,92}$=3.09, p=0.002), with regular nest owners (≥4 nesting events) possessing MHC haplotype No13.No18 under predation (SIMPER; p<0.001) or the rarer MHC haplotype No08.SCX15 (alleles No08 and SCX15, NCBI accession number AY687842 and EU541449, respectively) in the control group (Supplementary Table 4, SIMPER, p<0.001).

Interestingly, repeating the analyses described above for individuals with haplotype No13.No18 for those with the rarer haplotype No08.SCX15, revealed that individuals carrying this haplotype achieved a higher LRS (Figure 3D; $F_{1,86}$=6.46, p=0.013), increased final body condition ($F_{1,88}$=6.64, p=0.012) and overall lower parasite load (Figure 3C; $F_{1,86}$=7.89, p=0.006) in both treatments (Supplementary Table 5a-d). Males with No08.SCX15 also maintained more nests (Supplementary Table 5e; $F_{1,82}$=9.26, p=0.002). These results indicate that the functional advantage of specific MHC haplotypes underlies selection in both, parasite only and parasite and predator-exposed fish. But under predation, density changes may lead to changes in male competition over the course of the experiment, potentially removing reproductively active, i.e. conspicuous males from the breeding population. In addition, the LRS model revealed a stronger treatment by parasite load interaction

(Supplementary Table 5d; Figure 1C; $F_{1,87}$=5.09, p=0.027), reinforcing the argument for stronger parasite-mediated selection under control than predation condition.

**Discussion**

Predation and parasite infections are arguably among the most important biotic selection pressures, but how they interact to affect the evolution of host resistance remains largely unknown. Comparing host-parasite interactions in parasite only as well as parasite and predator-exposed populations of three-spined sticklebacks, we hypothesised that predation accelerates parasite-mediated coevolutionary dynamics, selecting against the most infected fish, i.e. those of poorest immunogenetic quality. Instead we found that only in the absence of pike predation, MHC haplotype frequencies correlated positively with individual parasite load and negatively with lifetime reproductive success. Rare and advantageous MHC haplotypes increased in frequency in the next fish generation, following predictions of negative frequency-dependent selection (NFDS). This is because fish carrying the relatively rare No08.SCX15 haplotype were less infected by parasites and achieved a higher lifetime reproductive success (LRS) under parasite-mediated selection. Under combined predation and parasite-mediated selection, a different dynamic was found: Overall MHC haplotype frequencies were neither associated with parasite load nor lifetime reproductive success. Only the most common MHC haplotype No13.No18 was linked to lower parasite load and higher LRS in the parental population and its frequency increased across generations within the predation exposed populations. This likely arose as a combination of parasite-mediated selection relaxed by predation removing infected and conspicuous individuals and reduced competition over nesting territories in an environment where host density was decreased by predation.

Under sole parasite-mediated selection, changes in MHC haplotype frequencies across generations followed patterns consistent with NFDS: individuals carrying common MHC haplotypes (i.e.

No13.No18) were more infected by parasites and achieved a lower LRS than individual with rarer haplotypes. Even though there are suggestions that rarity of MHC alleles alone could be advantageous (Bolnick and Stutz 2017; Phillips et al. 2018), it was also shown that being rare is not sufficient for a MHC allele to be associated with increased resistance (Eizaguirre et al. 2009*b*, 2012*b*). Here, individuals with very rare MHC haplotypes (<5% frequency) did not gain proportionally more LRS in either of the treatments. By contrast, individuals with resistance-associated MHC haplotype No08.SCX15 (~10% frequency) achieved a higher LRS and body condition than fish lacking this haplotype. This again demonstrates that rarity by itself is unlikely to be sufficient, but that the combination of rarity and functional advantage are the basis of NFDS.

While there is no need to invoke sexual selection to produce the observed NFDS pattern (Eizaguirre et al. 2012*a*, 2012*b*), in three-spined stickleback there is a strong case for MHC-based mate choice both for MHC compatibility and specific MHC alleles (e.g. Milinski et al. 2005, 2010; Andreou et al. 2017; Lenz et al. 2018). Females prefer less infected males that display their genetic quality by expressing more conspicuous and costly secondary sexual characters (Milinski and Bakker 1990) and build nests of higher quality (Figure 4A, Jäger et al. 2007). But are the patterns of NFDS modified under concomitant predation-mediated selection?

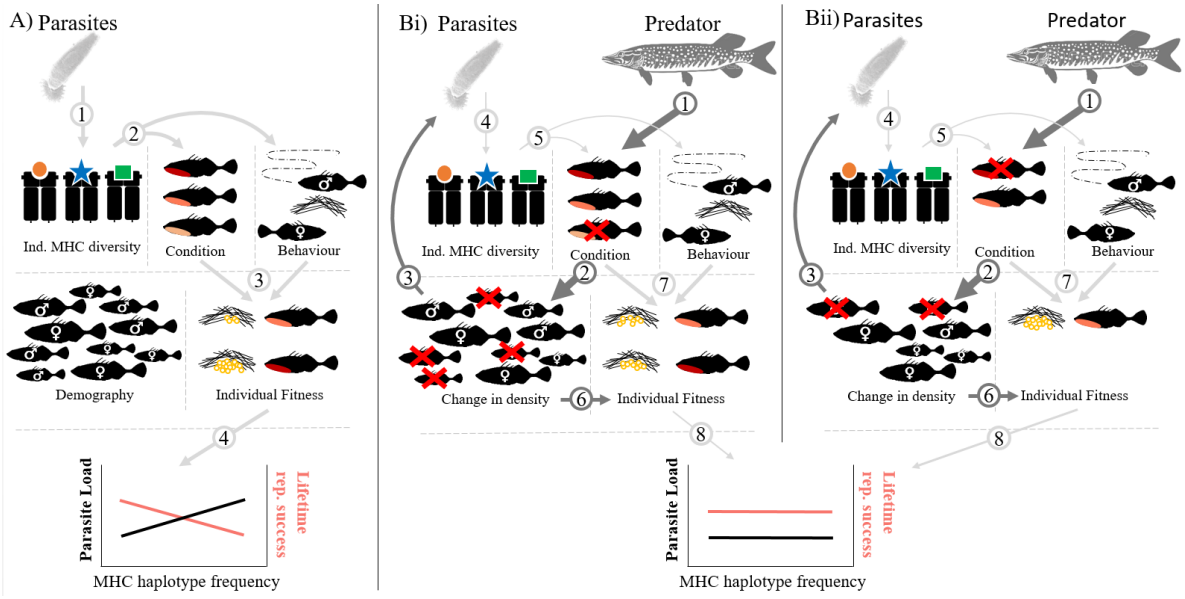


**Figure 4 A) (1) The strength of parasite-mediated selection and the presence of MHC haplotypes determine (2) host condition and behaviour (including mate choice, sexual display, foraging) with consequences for (3) reproductive success, favouring individuals with resistance MHC alleles and better fitness-related parameters. Rare MHC alleles often increase resistance and are associated with increased lifetime reproductive success. It results in (4) MHC frequency being positively associated with parasite load and negatively with lifetime reproductive success across the focal population matching prediction of the Red Queen dynamics. Bi) Under concurrent predation (1) prey condition decreases and highly infected individuals are removed from the population, (2) reducing overall host density. Bii) Predation will also remove some highly conspicuous individuals (1) who may carry resistance MHC alleles. Bi/ii) The change in density, in turn, affects (3) parasite transmission and prevalence, relaxing parasite-mediated selection (4-5) as well as (6) it impacts male competition over territories. This changes (7) parasite-mediated sexual selection dynamics of the host. As a consequence, the positive relationship observed between parasite load and MHC haplotype frequency under sole parasite-mediated selection erodes and together with the link between lifetime reproductive success and MHC haplotype frequency, affects (8) negative frequency dependent selection on MHC genes.**

Our experiment provides evidence that predation can alter host-parasite interactions, relaxing parasite-mediated negative-frequency dependent selection as predicted by Best (2018). Indeed, we found an overall lower individual parasite load in the predation treatment, where heavily infected

sticklebacks were removed by the pike because infected fish likely had poorer body condition, reduced vigilance and lessened escape capability (Crowden and Broom 1980; Milinski 1984, 1985). This lowered host population density (Figure 4Bi), changing parasite transmission rates (Arneberg et al. 1998) by removing possible parasite reservoirs as observed in Grant's gazelles (*Nanger granti*, Ezenwa and Worsley-Tonks 2018) or teleost fish like stickleback and their ecto-parasites (i.e. *Gyrodactylus* sp., Eizaguirre et al. 2009).

Changes in population density also alters reproductive success of territorial males (Lopez-Sepulcre and Kokko 2005; Eizaguirre et al. 2009*b*). Male sticklebacks display condition-dependent sexual ornaments (Milinski and Bakker 1990; Frischknecht 1993) and individuals with resistance MHC haplotypes display more conspicuous secondary sexual signals (e.g. red throat colouration), making part of this healthy population also the target of predation (Johnson and Candolin 2017, see Metz et al. 2018 for a similar effect in elk). Lower parasite pressure and lower population density resulted in relaxed parasite-mediated sexual selection as evidenced by a weaker relationship between parasite load and lifetime reproductive success. Specifically, fish with common MHC haplotypes (e.g. No13.No18) benefitted from the removal of highly competitive males relaxed parasite-mediated selection and reduced parasite load. This resulted in increased LRS and a larger contribution to the next generation than without predation (Figure 4Bii).

Our results are not independent of the nature of the predator. Pikes are ambush predators, taking prey size and behaviour into account when targeting a prey (Hart and Hamrin 1990). In response, three-spined sticklebacks use odour signals to avoid pike as well as inspection behaviour to determine its state of satiation (Steck et al. 1999; Häberli et al. 2005). This behaviour is vital for the prey to assess predation risk, but under infection anti-predator behaviour is altered (Milinski 1985; Aeschlimann et al. 2000). This compromises foraging behaviour (Milinski 1984, 1985) and we show it also extends to reproductive dynamics as density-mediated selection by predators affects male-male competition and parasite-mediated sexual selection.

Examples of NFDS selection on MHC diversity in nature are limited, and in general, the inclusion of predation when studying the dynamics of host-parasite coevolution is rare (Betts et al. 2016). Our study reports predation-induced changes in MHC frequencies over generations in vertebrates and corroborates predictions based on experimental invertebrate systems usually considered more amenable. For instance, a study on host-virus-predator dynamics (*Chlorella variabilis*-Chlorovirus PBCV-1-*Brachionus calyciflorus*) showed that host resistance evolution was significantly delayed when compared with the host-virus systems alone (Frickel et al. 2017). Similarly, coevolution between the bacteria *Pseudomonas fluorescens* and its virus SBW25Φ2 were altered due to the trade-off in host-resistance/prey-defence specialization when the predatory protist *Tetrahymena thermophile* was added (Friman and Buckling 2013). These findings, in combination with the results from the present study suggest that prey-predator and host-parasite relationships could be part of density-mediated eco-evolutionary feedbacks (Hiltunen and Becks 2014): parasite-mediated polymorphisms in resistance, for instance alleles of the MHC, might be limited by predation favouring more common alleles as a result of change in host density and relaxed parasite-mediated selection (Lazzaro and Little 2009; Huang et al. 2017).

Overall our experiment demonstrates that predators and parasites interact to shape prey/host evolution. While we expected predation to amplify the effects of parasite-mediated selection, if the predator had removed only heavily infected fish, our results illustrate density-dependent evolution of MHC-based resistance under concomitant predation. These outcomes may help explain why parasite-mediated negative frequency dependent selection is rarely observed in natural communities experiencing more than just selection by parasites.

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# CHAPTER 4. PREDATION MODIFIES PARASITE-MEDIATED MHC-BASED MATE CHOICE

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**Abstract**

Predation and parasitism are arguably the most important selective pressures and seem to be tightly linked: individuals susceptible to parasites are likely the primary target of predation. Predators and parasites hence affect population density but also prey morphology, foraging strategies and reduce reproductive success of their prey/hosts. Theoretically, mate choice for parasite resistance is an efficient mechanism to optimize selection for high quality males capable of resisting parasites and avoiding predators. The genes of the major histocompatibility complex (MHC) are a known genetic basis of parasite resistance and may therefore play an important role under predation pressure. We tested how predation affects infection intensity, lifetime reproductive success and MHC-based mate choice of three-spined sticklebacks. Within their native lakes, fish were stocked in enclosures either with or without a predatory pike. Weekly, eggs were collected and parenthood was determined for >14 000 eggs. Predation was biased towards males and highly infected sticklebacks. While MHC-based mate choice for compatibility and good genes were observed in the control group, under predation, mate choice solely for good genes was detected. We identified those good genes as resistance MHC alleles associated with increased lifetime reproductive success. Overall, under predation female mate choice operates a shift from optimal mate choice for compatibility and good genes, to a choice solely driven by good genes. Producing offspring with known resistance benefits may therefore be a more efficient strategy under concomitant predation than to rely on compatible partners with untested MHC haplotypes. As such this impacts the maintenance of MHC polymorphism and host-parasite coevolution.

## Introduction

Predation is one of the most important selection pressures for community and species' evolution (Krebs et al. 2001). Predators contribute to ecosystem stability (Allesina and Tang 2012), maintain species diversity (Meyer and Kassen 2007; Saleem et al. 2012) and shape evolutionary trajectories of their prey (Hiltunen and Becks 2014, Chapter 3). This is primarily attributed to the fact that predation decreases prey density (e.g. Wooster 1994; Connell 1998; Ripple et al. 2001) and favours the evolution of anti-predator traits, such as aposematic colouration in butterflies (Bates 1862) or spines in fish (Hoogland et al. 1956). But importantly, non-lethal effects of predation also create selective pressure (Cresswell 2008; Clinchy et al. 2013). The presence of a predator, for instance, can induce the growth of neck spines in *Daphnia pulex* (Krueger and Dodson 1981) and alter foraging behaviour in three-spined sticklebacks (Milinski and Heller 1978; Milinski 1993). Predation risk also impacts a prey's stress physiology, as found for snowshoe hares, which experience high cortisol levels and poor body condition in years of high predator prevalence (Sheriff et al. 2011; Clinchy et al. 2013). This perceived risk of predation can ultimately affect reproductive success of the prey (Zanette et al. 2011).

Interestingly, predation risk is not independent of other selection pressures, specifically parasitism. Firstly, highly infected individual prey are the primary targets of predation due to reduced fitness (Milinski 1985; Lafferty and Morris 1996). Secondly, increased predation may be the result of parasite manipulation of the prey to maximize transmission rates of parasites with complex life cycles (Hammerschmidt et al. 2009; Dianne et al. 2011). This pattern may further be modified under multiple infection (Hafer and Milinski 2016), which is the common state in nature. Parasite infection also lowers foraging efficiency and vigilance (e.g. Milinski 1985) and reduces anti-predator responses (Blake et al. 2006). Lastly, both predation and parasitism decrease the expression of condition-dependent sexual ornaments (Milinski and Bakker 1990; Ruell et al. 2013) and alter mate choice (Aeschlimann et al. 2003; Johnson and Basolo 2003), extending the effects of those natural selection pressures to sexual selection.

Overall, there should be evolved mechanisms to jointly optimize predation avoidance, increase parasite resistance and maximize reproduction. Mate choice is such a hypothetical mechanism. Specifically indirect benefits, such as from compatible and good genes, reflect a genetic component of the choice (Andersson 1994). In mate choice based on compatible genes, females do not seek males of particularly high quality, but rather choose males which combined with their own genetic make-up will result in offspring of increased quality (Tregenza and Wedell 2000; Reusch et al. 2001*a*; Milinski et al. 2005). Conversely, heritable traits with measurable fitness benefits are encoded by good genes (Andersson 1994). Such good genes permit the expression of costly sexual ornaments (Hamilton and Zuk 1982; Milinski 2014).

To date, the genes of the major histocompatibility complex (MHC) are the best known genetic basis of resistance and mate choice (Janeway et al. 2005; Kamiya et al. 2014; Milinski 2014). The MHC genes encode a suite of structurally related yet distinct molecules, which present either self-peptides or peptides derived from phagocytosed pathogens to T-lymphocytes. This, in turn, mounts specific immune responses against parasites and pathogens (Janeway et al. 2005). MHC class II$\beta$ genes, in particular, induce immune responses against extracellular parasites (Janeway et al. 2005).

In nature, parasite-mediated balancing selection maintains the extraordinary high polymorphism in the MHC genes (Eizaguirre and Lenz 2010; Spurgin and Richardson 2010). In a variety of jawed vertebrates sexual selection aids in the maintenance of MHC polymorphism: MHC-based mate choice targets specific good and/or compatible MHC genes (Milinski 2006, 2014; Kamiya et al. 2014). Good genes are resistance MHC alleles against the currently most prevalent pathogens allowing males to allocate resources to costly sexual signals (Milinski 2014). MHC-compatibility is predictably achieved through olfaction (Milinski et al. 2005) with males producing a costly odour-based signal that is recognised by females (Milinski et al. 2010), aiming to achieve optimal individual MHC diversity and increased offspring resistance (Reusch et al. 2001*a*; Milinski et al. 2005; Chaix et al. 2008).

In a theoretical scenario, under predation parasite-resistant individuals are likely to compete more successfully for territories and mating opportunities (Fox and Hudson 2001), attract more mates (Milinski and Bakker 1990; Bakker and Mundwiler 1992), and ultimately, produce more and fitter offspring. Yet, the interactive effect of parasites and predators on MHC-based mate choice and its consequences for host-parasite dynamics are unknown.

The three-spined stickleback (*Gasterosteus aculeatus*) is host and prey to various parasites and predators respectively. Females predictably choose males with compatible MHC alleles to optimise individual MHC diversity (Reusch et al. 2001*a*; Aeschlimann et al. 2003; Milinski et al. 2005) and increase parasite resistance for their offspring (Wegner et al. 2003*a*). Moreover, choice based on costly sexual signals, such as the carotenoid-based throat colouration, indicates body condition (Milinski and Bakker 1990; Bakker and Mundwiler 1992), parasite load (Milinski and Bakker 1990) and identifies males with specific good genes (Jäger et al. 2007; Eizaguirre et al. 2009*b*, 2012*b*). Under sole parasite-mediated selection, MHC-based mate selection for compatible and good genes results in an increased individual lifetime reproductive success (Eizaguirre et al. 2009*b*; Kalbe et al. 2009; Lenz et al. 2009*b*).

Here, wild-caught three-spined sticklebacks were placed in twelve semi-natural enclosures (over 2 consecutive years) within their native lake, which allowed for natural behaviours of the fish and parasite exposure. Each year, half of the enclosures were stocked with a predatory pike (*Esox lucius*, 20-30cm) – a natural predator of sticklebacks. Parenthood analysis on eggs was used to determine female mate choice. We predicted female choice for both good and compatible genes under sole parasite-mediated sexual selection in the control group (Milinski et al. 2005; Eizaguirre et al. 2009; Kalbe et al. 2009; Lenz et al. 2009*b*). Under risks of predation, foraging performance as well as anti-predator behaviours (Milinski and Heller 1978; Milinski 1993) should reduce food intake, lowering overall body condition and growth (Aeschlimann et al. 2000). Parasites should amplify this pattern since parasitized stickleback forage at greater risk, lower effectiveness, are less vigilant and have a

lower escape response (e.g. Milinski 1985; Külling and Milinski 1992; Blake et al. 2006). Therefore, we expect natural and sexual selection against parasitized individuals, leaving sticklebacks carrying resistance MHC alleles against predominant parasites as well as those carrying themselves the optimal individual MHC diversity gaining fertilisation and increasing their lifetime reproductive success.

**Material and Methods**

*FISH COLLECTION AND EXPERIMENTAL DESIGN*. In April 2011 and 2012, three-spined sticklebacks were caught from the lake Großer Plöner See (54°9'21.16'N, 10°25'50.14'E, Germany). After capture, a small spine clip was taken from all fish for DNA extraction and later, individual identification. Fish were measured, weighed and randomly distributed across six groups, each containing twelve males and twelve females. Each group was then released into one of six stainless steel enclosures (3 x 3m, height of 1m, 0.4-0.6m above the water surface, 0.5cm mesh size) within their lake of origin two weeks later (Kalbe et al. 2009). The mesh of the enclosures allows for free passage of all parasites and prey items. The enclosures also contained structural elements (stones, plants, wooden debris, etc.) used by male sticklebacks for nest-building and by both sexes for shelter. For the purpose of this experiment, in each year, three of the six (2011: Enclosure 1, 3, and 5; 2012: Enclosure 2, 4, and 6) enclosures were stocked with a Northern Pike (*E. lucius*, 30-40cm). Each enclosure was protected against avian predation by a net.

*EGG COLLECTION*. On a weekly basis, all enclosures were inspected for nests. Egg clutches were carefully removed from each nest (Kalbe et al. 2009). Twenty-four randomly picked eggs of each clutch were used for DNA extraction and later parenthood analysis. Extraction took place on a Freedom evo robot (Tecan) using Invisorb Tissue HTS 96 kit / R (Stratec). A total of 14 742 eggs were genotyped for parenthood analysis based on nine microsatellites (Kalbe et al. 2009).

*RE-CAPTURED PARENTAL FISH, MICROSATELLITES AND MAJOR HISTOCOMPATIBILITY COMPLEX-TYPING*. At the end of the experiment, all surviving fish were recaptured, dissected and

screened for parasites (Eizaguirre et al. 2009*b*; Kalbe et al. 2009; Lenz et al. 2009*b*). At this stage, fish were measured to estimate body condition – calculated as $(\frac{weight}{length})^b$ with $b$ representing the regression coefficient calculated from the logarithm-transformed values of weight and length (Frischknecht 1993) - and fin clipped to associate reproductive success with individual characteristics at the end of the experiment.

DNA extractions, from both dorsal spines (before the experiment) and fins (after the experiment), were performed using DNeasy 96 Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. All fish were genotyped for nine microsatellites, combined in two different multiplexed PCR, both before release in the enclosure and after recapture for re-identification (Eizaguirre et al. 2009*b*; Kalbe et al. 2009).

The MHC class II$\beta$ diversity in all parental fish was determined using reference-strand-mediated conformation analysis (RSCA) optimised for the three-spined stickleback (Lenz et al. 2009*a*). The target was the exon II of the MHC gene, which encodes the highly variable peptide-binding region of the beta chain of the MHC molecule (Lenz et al. 2009*a*). Noteworthy, the MHC II$\beta$ genes in stickleback are duplicated and frequently occur in tightly linked haplotypes (Lenz et al. 2009*a*). Even though variants may stem from paralogs, we refer to them as 'alleles'.

*PARENTHOOD ANALYSIS AND MALE NESTING OR SNEAKING STRATEGIES.* Parenthood assignments of all sampled eggs from 2011 and 2012 was performed with the software PAPA (Duchesne et al. 2002) and allowed to determine the individual lifetime reproductive success of all females that entered the experiment (LRS, Kalbe et al. 2009). The results of the parenthood analysis were also used to assign male mating strategies: Males that sired the majority of the eggs within in a clutch were classified as nest owners. All eggs sired by the nest owner were assumed to be the outcome of active female mate choice. Eggs were considered to be sired by sneaker males when genotyping revealed that another male than the nest owner fertilised them. Sneaking is not the result

of female choice. When the egg was neither assigned to the nest owner nor other eggs from the same female were collected, the egg was categorised as stolen from another nest (Kalbe et al. 2009). The assignment of such strategies has previously been verified (Kalbe et al. 2009). Based on this assignment we could calculate individual lifetime reproductive success.

*DATA ANALYSES*. All statistical analyses and graphical visualisations were performed with R version 3.3.1 (R Core Team 2016). Model residuals were checked for normality and homoscedasticity and data were transformed if required to meet test assumptions. All models were backward-selected using the *anova* function.

*SURVIVAL*. A generalised linear model was used to compute the likelihood of survival (i.e. binomial parameter) using treatment, sex, initial body condition, individual MHC diversity and all two-way interactions with the predation treatment as fixed factors and year and enclosure set as random factors.

*MHC DIVERSITY AND BODY CONDITION*. We used Wilcoxon rank-sum test to assess the effects of year and sex on MHC allele number and Kruskal-Wallis rank test to estimate variation between enclosures within years. Another Wilcoxon rank-sum test was run to compare individual MHC allele number between treatments across years. MHC variant pools were compared using an Analysis of Similarity (ANOSIM) between treatments across years. The results highlight an even individual MHC diversity and allele pool across the entire experiment and thus, justify data pooling (all results see Supplementary Table S1). Similarly, we confirmed with a linear mixed effect model with treatment as a fixed and enclosure and year set as random factors, that no bias with respect to initial body condition was introduced into the enclosures at the start of the experiment between treatment groups ($F_{1,10}$=0.154, p=0.703).

*PARASITE COMMUNITY*. First, a PERMANOVA was used to test whether parasite communities differed between treatments nested within year. Individual parasite species abundance

and diversity were combined into an 'Individual Parasite Index' ($I_{PI}$, Kalbe et al. 2002). Then, the $I_{PI}$ (log-transformed)was analysed using a single mixed effect model with enclosure and year as random factors to estimate the effects of the predation treatment, sex, initial body condition and individual MHC diversity as well as all possible two-way interactions with the predation treatment. A second model was run with the quadratic term of individual MHC diversity to investigate whether we can observed a quadratic relationship between individual MHC diversity and parasite load. Second, an analysis of similarity percentage (SIMPER) was run for both years independently to estimate those parasite species cumulatively explaining 90% of the variance in parasite load across treatments and years (Supplementary Table S3). Then we estimated the effect of treatment and frequent MHC haplotypes (>10%, Supplementary Table S5), initial body condition and their two-way interaction within each year on those identified parasite species (log-transformed) with a mixed effects model. Enclosure was set as a random factor.

*REPRODUCTIVE OUTPUT AND MALE FERTILISATION STRATEGIES*. Reproductive output in terms of number of eggs recovered from the enclosures was compared between treatments with a $\chi^2$-test. We compared the proportions of fertilisation success of each male fertilisation strategy with a Welch T-test between treatments. Then, we calculated the individual proportion of eggs sneaked with respect to the total eggs sired by each male (Eizaguirre et al. 2009*b*). Arcsine-square root transformed proportion of sneaked eggs was then analysed using a mixed effects model with treatment, LRS, initial body condition, $I_{PI}$ and individual MHC diversity as well as all two-way interactions with predation treatment as fixed factor and enclosure and year as random effects.

*LIFETIME REPRODUCTIVE SUCCESS AND MATE CHOICE.* Square-root transformed LRS entered a mixed effect model using predation treatment, sex, initial body condition, individual MHC diversity, $I_{PI}$ and all two-way interactions possible with predation. Year and enclosure were set as random effects. LRS is mostly the result of mate choice, hence, to estimate the degree of self-

reference females used during mate choice, the MHC variant-sharing index was determined between females and males from the same enclosure in each year (Eizaguirre et al. 2009*b*):

$$D = \frac{2F_{ab}}{F_a + F_b}$$

where $F_{ab}$ is the number of MHC variants shared and $F_a$ and $F_b$ is the sum of MHC variants of individuals *a* and *b*, respectively. Subsequently, we simulated a 1000 random female mate choice events with respect to MHC variant sharing value among all males that had been classified as being reproductively active, i.e. found to fertilise eggs in a given week. The same was done for the observed mate choice events. Wilcoxon rank-sum tests (1000 repeats) were used to compare the observed MHC variant-sharing value against that of random choice in each enclosure and each year. Similarly, Kolmogorov-Smirnov tests were computed to compare observed and simulated distribution (Eizaguirre et al. 2009*b*). In both cases, the total number of significant tests ($p< 0.05$) were used to determine whether mate choice was non-random with regards to MHC (Supplementary Table S9). This means when repeating this comparison of random versus observed mate choice 1000 times and the total number of significant tests were beyond 950 (i.e. <0.05) we accepted mate choice to be non-random. In a second step, to determine the direction of MHC-based mate choice, i.e. similar or dissimilar MHC diversity, we compared the observed MHC variant sharing values with simulated choice for most MHC-dissimilar genotypes in each enclosure using both the Wilcoxon rank-sum and Kolmogorov-Smirnov tests (1000 repeats each). Lastly, the same analyses were performed to investigate whether mate choice was random with regards to relatedness estimated from nine microsatellites. This information was then combined with a binomial test to test for consistency of the observed patterns across enclosures and years. Since the breeding period had two reproductive peaks (Supplementary Figure 1 A), we repeated the analysis for an early (1-3 weeks) and late (4-7 weeks) reproductive period.

**Results**

*SURVIVAL, AND PARASITE LOAD AND COMMUNITY.* After 39 and 54 days, a total of 69 (N♀=34, N♂=35) and 65 (N♀=35, N♂=30) individuals were recovered from the control enclosures in 2011 and 2012, respectively. Twenty-seven individuals (N♀=15, N♂=12) in 2011 and 24 (N♀=17, N♂=7) in 2012 survived in the predation enclosures. Mortality was significantly higher in the predation treatment ($F_{1,285}$=10.30, p<0.001) and fewer males than females survived ($F_{1,285}$=10.77, p=0.001, Supplementary Table S2). Initial body condition and individual MHC diversity had been removed during model selection.

Parasite communities did not differ between treatments (PERMANOVA, $F_{1,180}$=1.617, p=0.135), but showed differences in community between years (PERMANOVA, $F_{2,180}$=10.633, p<0.001). The cumulative dissimilarity in parasite communities between years (90.2%) was driven by the ciliates *Apiosoma* sp. (36.4%; 2011: 162.1 (±39.0SE=standard error); 2012: 214.2 (±39.9SE)) and *Trichodina* sp. (23.1%; 2011: 109.5 (±35.2SE), 2012: 118.0 (±28.9SE)), digenean trematode *Diplostomum* sp. (8.2%; 2011: 48.5 (±3.3SE); 2012: 32.6 (±2.6SE)), the cestode *Valipora campylancristrota* (7.6%; 2011: 25.9 (±2.9SE); 2012: 0.7 (±0.1SE)), the trematode *Echinochasmus* sp. (5.4%; 2011: 12.6 (±1.3SE); 2012: 18.1 (±2.8SE)), the monogenean *Gyrodactylus* sp. (4.8%; 2011: 13.6 (±1.9SE); 2012: 10.8 (±2.1SE)) and the trematode *Cyathocothyle prussica* (4.7%; 2011: 18.0 (±1.8SE); 2012: absent; see also Supplementary Table S3).

Yet, we found that the $I_{PI}$ was significantly lower in surviving fish from the predation treatment compared to control ($F_{1,140}$=6.58, p=0.011; Figure 1A). Furthermore, $I_{PI}$ negatively correlated with initial body condition and more so in the control than in the predation treatment (treatment * body condition, $F_{1,167}$=4.73, p=0.031, Supplementary Table S4). Neither sex nor individual MHC diversity was correlated with $I_{PI}$. Substitution of individual MHC diversity with its quadratic term did not alter the results.

Investigating the link between specific MHC alleles and parasite abundance, we found that the presence of haplotype No08.SCX15 (alleles No08 and SCX15, NCBI accession number AY687842 and EU541449, respectively) was associated with higher resistance towards *V. campylancristrota* infection (Supplementary Table S5). Furthermore, carriers of haplotype No01.No12 (alleles No01 and No12, NCBI accession number DQ016399 and DQ016499, respectively) were more susceptible to *Gyrodactylus* sp. in 2011 (Supplementary Table S5). In contrast, only haplotype No13.No18 (alleles No13 and No18, NCBI accession number AF395711 and AY687846, respectively) conferred a slightly higher resistance against *Echinochasmus* sp. in 2012 (Supplementary Table S5). Given these results, we substituted individual MHC diversity in the original $I_{PI}$ mixed effect model with those specific MHC haplotypes within 2011 and 2012, respectively, keeping treatment, initial body condition and sex as fixed variables, and enclosure as a random factor. The results demonstrate that haplotype No08.SXC15 was also associated with increased overall parasite resistance regardless of treatment, sex or body condition in 2011 (Figure 2A; $F_{1,87}$=7.99, p=0.006; Supplementary Table S4). Equally, haplotype No01.No12 was associated with overall susceptibility ($F_{1,86}$=5.03, p=0.028; Supplementary Table S4). Haplotype No13.No18 had no effect on overall parasite load in 2012.

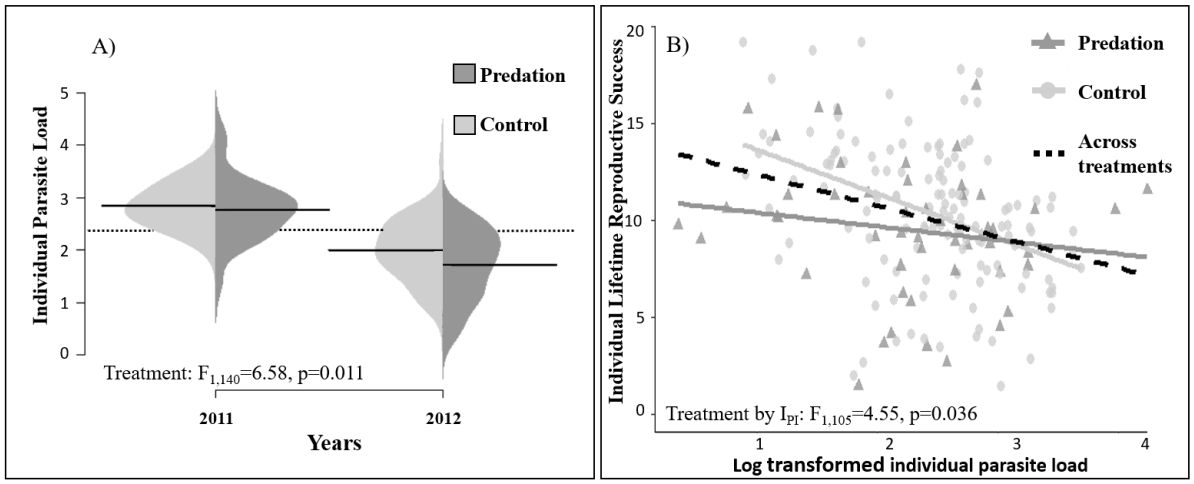


**Figure 1. A) Parasite load ($I_{PI,}$ log-transformed) is shown for fish under control (light grey) and predation (dark grey) for both experimental years. The graphic shows overall parasite load (dotted line), the distribution of parasite load, and the mean parasite load within treatment and year (long, black horizontal lines). B) Relationship between lifetime reproductive success (LRS, square root transformed) and parasite load (log-transformed) for fish in the control group (light grey circle) and under predation (dark grey triangle).**

Interestingly, haplotype-related specific resistance effects could stem either from the sole presence of the haplotype (Eizaguirre et al. 2009*b*; Lenz et al. 2009*b*; Eizaguirre et al. 2012*b*) or from it belonging to the optimal MHC diversity (Milinski 2014). To decipher both effects, we ran a generalised linear mixed effect model on the presence or absence of the specific haplotypes as a function of individual MHC diversity. Neither haplotype No08.SCX15 (3: z=-0.21, p=0.834; 4: z=1.56, p=0.118; 5: z=0.69, p=0.489) nor haplotype No01.No12 (3: z=-0.92, p=0.355; 4: z=-0.13, p=0.899; 5: z=-1.08, p=0.280) were more common at intermediate diversity than expected by chance suggesting beneficial effects associated with the presence of the haplotype itself. Furthermore, following the hypothesis that more divergent MHC alleles may confer a higher resistance advantage (Wakeland et al. 1990), we investigated whether the amino acid-based p-distance between the alleles of each haplotype was divergent when compared with the average range of pairwise comparisons within the population. We found that neither haplotype No08.SCX15 nor No01.No12 were significantly more divergent (Supplementary Figure 2).

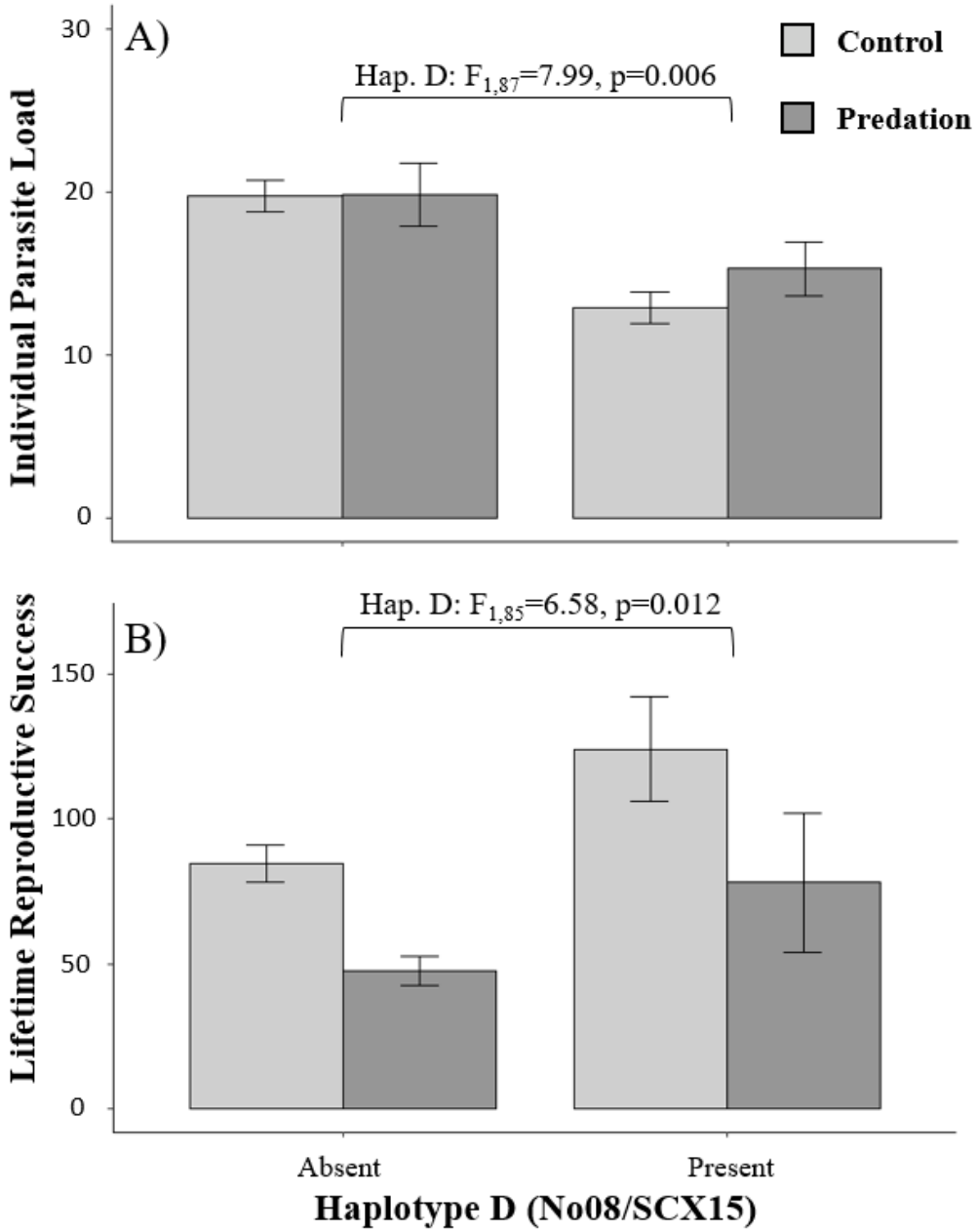


**Figure 2. A) Mean parasite load ($I_{PI}$, ±SE) of individuals with and without the resistance haplotype No08.SCX15 for under control (light grey) and predation (dark grey) conditions. B) Mean the lifetime reproductive success (LRS, ±SE) for fish with and without the resistance haplotype No08.SCX15**.

*LIFETIME REPRODUCTIVE SUCCESS.* Parenthood analysis was completed for a total of 101 (5.584 eggs) and 176 clutches (9.158 eggs) in 2011 and 2012, respectively. Ambiguous assignments

(~6%) were removed from further analysis. As expected, a $\chi^2$-test revealed that fewer eggs were recovered from predation treatment ($x_5^2$=1335.8, p<0.001) - a result of the reduction in fish density. A total of 77.7% (±2.2 SE) and 81.6% (±1.7 SE) of all eggs in the control and predation treatment, respectively, were assigned to nest owners (t-test: *d.f.*=67, t=-1.35, p=0.181).

Individual lifetime reproductive success (LRS) was lower in the predation treatment ($F_{1,19}$=5.99, p=0.025) and decreased with increasing $I_{PI}$ ($F_{1,105}$=4.33, p=0.040; Figure 1B; Supplementary Table S6). This relationship, however, was weaker under predation treatment resulting in a treatment by $I_{PI}$ interaction ($F_{1,105}$=4.55, p=0.036; Figure 1B). Sex, individual MHC diversity and initial body condition were not correlated with LRS (Supplementary Table S6). Exchanging individual MHC diversity by the presence/absence of the resistance MHC haplotype No08.SCX15 in the 2011 model, we found that individuals with haplotype No08.SCX15 had increased LRS independently of the treatment group ($F_{1,85}$=6.58, p=0.012; Figure 2B; Supplementary Table S6). In this model, the interaction between $I_{PI}$ and treatment remained ($F_{1,86}$=3.89, p=0.052). Moreover, LRS seemed to increase with higher body condition under predation only (treatment * initial body condition; $F_{1,87}$=3.36, p=0.070). On the other hand, individuals with haplotype No01.No12 experienced reduced reproductive success ($F_{1,83}$=4.35, p=0.040; Supplementary Table S6). The model also uncovered a treatment by $I_{PI}$ interaction ($F_{1,86}$=4.90, p=0.030) and showed that increased initial body condition was positively associated with individual LRS under predation pressure ($F_{1,87}$=4.51, p=0.037). From those two results, it is clear that when considering MHC haplotypes, the addition of fish body condition improves the models and reveals the importance of this trait under predation. No effect of specific MHC haplotypes was established on LRS for 2012.

The number of eggs fertilised by sneaking was strongly negatively correlated with LRS ($F_{1,68}$=36.42, p<0.001) and fewer eggs were sneaked under predation ($F_{1,26}$=7.46, p=0.011; Supplementary Table S7). Furthermore, the negative relationship between LRS and the number of eggs fertilised through

sneaking was weaker under predation than in the control group (treatment * LRS: $F_{1,67}$=10.09, p=0.002). Replacing individual MHC diversity with haplotypes No08.SCX15 and No01.No12, we found that LRS (expressed as residuals of the regression with specific haplotypes) remained highly predictive of the number of eggs sneak-fertilised (model with No08.SCX15: $F_{1,40}$=28.70, p<0.001; model with No01.No12: $F_{1,40}$=20.66, p<0.001; Supplementary Table S7). Additionally, the individuals with haplotype No08.SCX15 sneaked fewer eggs ($F_{1,40}$=4.93, p=0.032), whereas individuals with haplotype No01.No12 gained more fertilisation success by sneaking ($F_{1,40}$=17.14, p<0.001). Moreover, in both models fertilisation gained by sneaking increases with initial body condition ($F_{1,40}$=4.55, p=0.039; $F_{1,40}$=6.67, p=0.014; Supplementary Table S7).

*FEMALE MATE CHOICE – COMPATIBLE GENES*. Over the entire course of the experiment and for the 6 replicated populations per treatment, female mate choice appeared to be random with regards to MHC sharing value, i.e. compatibility (See Supplementary Table S8A, binomial test; control: p=1; treatment: p=0.688) and relatedness measured from nine microsatellites (See Supplementary Table S8B, binomial test, control: p=0.219; treatment: p=1).

When focusing only on the early breeding period, random choice with regards to relatedness was observed, but mate choice for compatibility was detected in all 6 control enclosures (binomial test, p=0.031, Supplementary Table S8A). In the predation treatment, compatible MHC-dependent mate choice was observed in 5 out of the 6 enclosures (binomial test, p=0.219; Supplementary Table S8A).

With subsequent Wilcoxon sum-rank and Kolmogorov-Smirnov tests we found that during the early period, female sticklebacks chose consistently more MHC-dissimilar males than by random (W=453690, p<0.001), but were not aiming at maximising MHC diversity (D=0.092, p<0.001; Figure 3). We, therefore, conclude that females search for males combined with which they would achieve an intermediate MHC diversity in their offspring (Eizaguirre et al 2009).

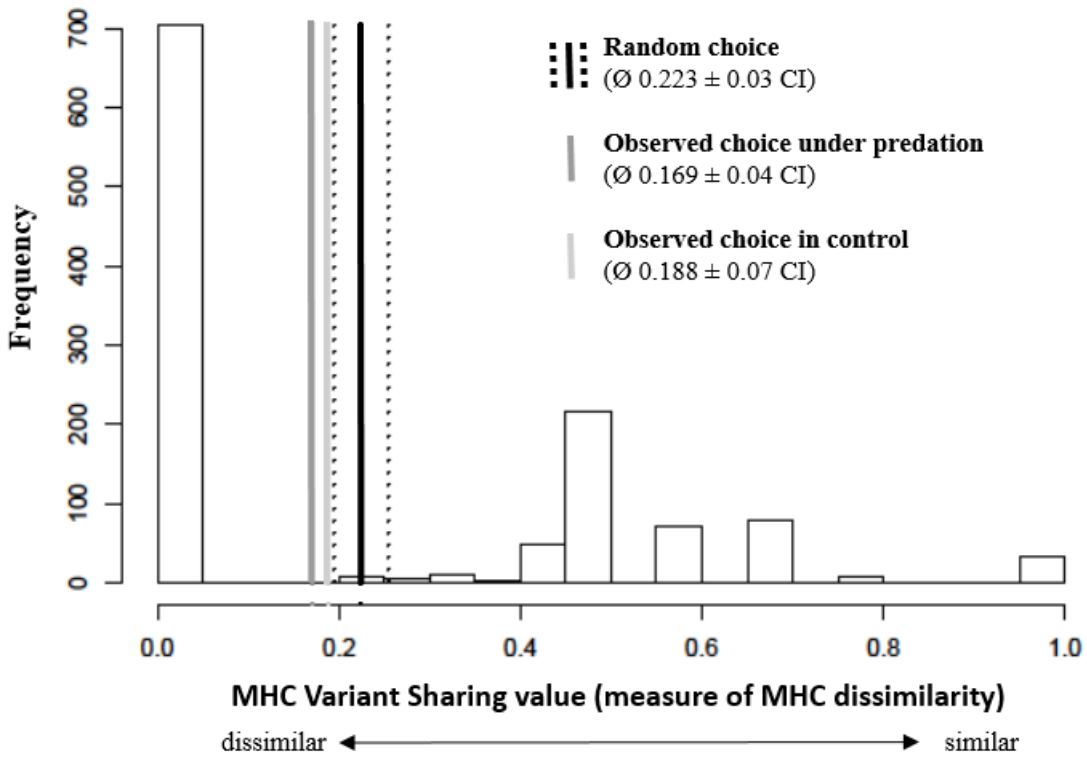


**Figure 3. Mean MHC variant sharing value (measure of MHC dissimilarity) in control (light grey) and predation (dark grey) is lower compared to the mean value derived from random mating (black with dotted lines indicating 95% CI) during the first part of the breeding season (week 1-3).**

*FEMALE MATE CHOICE – GOOD GENES*. Since haplotype No08.SCX15 was a strong predictor of reproductive success in 2011, we calculated in a generalised linear mixed effect model with enclosure as random factor whether the males carrying haplotype No08.SCX15 had a higher likelihood of being chosen (Supplementary Table S9). We found that throughout the entire breeding period and within each of the breeding periods in 2011, males with haplotype No08.SCX15 were more frequently chosen by females than individuals lacking this haplotype ($z=3.24$, $p=0.001$). Under predation, however, females prefer males lacking this haplotype No08.SCX15 (haplotype * treatment: $z=-3.66$, $p<0.001$).

**Discussion**

Predation is a major natural selection pressure that interacts with other selective agents, such as parasites (e.g. Milinski 1985; Lafferty and Morris 1996). Here, we hypothesized that there should be evolved mechanisms to jointly optimize predation avoidance, increase parasite resistance and maximize reproduction in a fish species. We speculated mate choice to be such a functional link. Focusing on MHC genes - the best known genetic basis of resistance and mate choice in jawed vertebrates - we reveal that predation directly affected the stickleback populations in two ways: First, predation was male biased with a 26% survival chance for males, compared with 44% for females. By contrast, 96% and 92% of the females and males respectively survived in the control group. Second, fish from the predation treatment showed a lower parasite load than those from the control group. It is, therefore, evident that predation pressure altered population dynamics and that selection by predators and parasites is tightly linked in complex ecosystems. Moreover, we were unable to detect MHC-based mate choice for compatibility under predation, but in the control, at least, during the early breeding period. Female choice for genes of MHC haplotypes with functional advantages was observable throughout the breeding season and regardless of treatment. These results emphasise that predation directly impacts population dynamics and parasite-mediated selection with likely indirect consequences for sexual selection in the prey/host.

We found increased predation on males, likely resulting from increased costs of expression of conspicuous sexual signals, such as the red throat colouration (Johnson and Candolin 2017). A trade-off between survival and reproductive advertisement in males is common. In a classic experiment, for instance, spot size and colour in male Trinidadian guppies, *Poecilia reticulata*, determined both, female choice and predation risk by the pike cichlid, *Crenicichla alta* (Endler 1980). And in the ground-dwelling wolf spider, *Hygrolycosa rubrofasciata*, acoustic signalling to attract female attention simultaneously increases risk of predation (Kotiaho et al. 1998). It is interesting to note that this trade-off persist in our study even though male sticklebacks are known to minimise courtship and

dangerous fertilisation strategies when faced with a predator (Candolin 1997, 1998). These attempts to conceal conspicuousness, underscore the severity of the survival-reproduction trade-off experienced by males in the presence of predators (Hughes et al. 2012).

In our experiment we also observe reduced parasite infection load under predation. This result suggests that predation pressure removes highly infected individuals from the population (Best 2018), likely as a by-product of decreased host condition due to the costs of parasitism. This is emphasised by the negative correlation between body condition and parasite load which is known to correlate with poor anti-predator behaviours of infected individuals (Milinski 1985, 1993; Lafferty and Morris 1996; Blake et al. 2006). But what are the consequences for mate choice and lifetime reproductive success?

Under parasite-mediated selection, theory predicts that females choose males with compatible genes in order to achieve an optimal MHC diversity for their offspring (reviewed in Milinski 2014). Across the two years of the experiment, we confirmed this hypothesis, particularly early on in the breeding season. Later on, sequential mate choice (Milinski and Bakker 1992; Eizaguirre et al. 2009*b*) and male final reproductive investment (Eizaguirre et al. 2009*b*; Kalbe et al. 2009) may have altered mate choice, hiding the pattern. On the contrary, MHC-based mate choice for specific MHC alleles was observable throughout the entire breeding season with males having an increased likelihood of being chosen when carrying the resistance haplotype No08.SCX15. Under sole parasite-mediated selection we hence confirm previous results and demonstrate that females use both compatibility and good genes to make their mating decision (Kalbe et al. 2009; Eizaguirre et al. 2009, Lenz et al 2009). Such results serve as positive control in our experiment.

Under both, predation and concurrent parasite-mediated selection we found more variation with regard to MHC-based mate choice for compatibility. Since female mate choice for compatible MHC alleles uses self-reference, the likelihood to find a mate offering an optimal MHC composition for the

offspring (Reusch et al. 2001*a*; Aeschlimann et al. 2003; Milinski et al. 2005) decreases with fewer males to choose from (Eizaguirre et al. 2009*b*). The lack of compatibility combined with a low male density under predation may therefore illustrate the importance of a large population size for this type of mate choice (Wacker et al. 2013). On the other hand, we found that females mated more often with males carrying the resistance MHC haplotype No08.SCX15 at any given time than expected by chance. At the same time, fish carrying the haplotype No08.SCX15 may become a main target of predation, because individuals with this haplotype experience enhanced resistance, leading likely to more conspicuous sexual displays (i.e. good gene, Andersson 1994). Nevertheless, individuals with No08.SCX15 sired more offspring demonstrating the extended benefits of MHC-mediated resistance with respect to predator avoidance and revealing that good genes functionally link natural and sexual selection.

Investigating the frequency of individual fertilisation strategies, we found that males sneaked less under predation (~10%) than control (~13%). This was expected, since males reduce risky fertilisation tactics under threat of predation (Candolin 1998). But interestingly, the number of eggs fertilised by sneaking was negatively correlated with lifetime reproductive success, albeit weaker under predation. Moreover, males with the resistance haplotype No08.SCX15 sneaked less often than individuals lacking this haplotype. By contrast, individuals with the susceptibility haplotype No01.No12 increased their reproductive success through sneaking behaviour. Yet, within this group of sneaker males, the largest ones gained more fertilisation, demonstrating the advantage of size under risks of predation. These findings are in line with the idea that high-quality males should be more cautious to secure future reproductive success (Engqvist et al. 2014), but differ from some earlier work that suggested that individuals with high mating success will also resort to risky alternative fertilisation tactics (Candolin 1998; Eizaguirre et al. 2009*b*).

So how did mate choices and alternative fertilisation tactics translate into lifetime reproductive success? In the control group, individual lifetime reproductive success was negatively correlated with

individual parasite load demonstrating, once more, the evolutionary costs of parasitism (Eizaguirre et al. 2009*b*; Kalbe et al. 2009). Under predation, this measure of Darwinian fitness was independent of parasite load. Non-random predation on highly infected individuals with low reproductive potential creates an environment of relaxed parasite-mediated sexual selection with fewer mates where initial body condition becomes the primary determinant of lifetime reproductive success. This is shown by the interaction between initial body conditions and treatment on lifetime reproductive success: for fish of high initial body condition, which relates to fast growth, lifetime reproductive success was similar between treatments. This follows the prediction that larger fish escape predation better and gained increased fertilization (Milinski and Heller 1978; Zuk and Kolluru 1998). Hence, under predation, reproductive success is determined by traits, such as size and body condition, favouring escape and anti-predator responses (Milinski 1985; Külling and Milinski 1992).

Taken all together, our results reveal that predation links natural and sexual selection through MHC genes and impacts the maintenance of its polymorphism. Predation eliminates unfit individuals from the population or from the breeding pool, reducing diversity and density. Furthermore, predation favours males with specific good genes providing resistance under the current parasite pressure. This way, predation further reduces MHC diversity available to female choice. Predation may therefore impair the role of mate choice in the maintenance of MHC polymorphism with consequences for host-parasite coevolution (Chapter 3). Interestingly yet, we find that sneaking by relatively less fit males plays an underestimated role in supplying genetic variation.

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# CHAPTER 5. SEASONAL VARIATION IN PARASITE-MEDIATED SELECTION AND MHC-BASED RESISTANCE

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**Abstract**

Seasonality is one of the most pervasive drivers of environmental variation, yet, how it impacts fitness remains elusive. Parasite diversity and abundance are likely to be important agents of selection for resistance within a generation and across seasons, impacting population dynamics. We exposed 780 laboratory-reared sticklebacks with distinct Major Histocompatibility Complex (MHC) haplotypes, to variable parasite selection during spring, summer and autumn. As expected, parasite diversity and abundance differed among seasons and was highest during summer. Several MHC haplotypes were associated with either high or low parasite infection: MHC Haplotype No05 was, for instance, associated with increased resistance to the eye fluke *Diplostomum* sp. - a species prevalent throughout the year. Individuals with haplotype No05 had consistently higher body condition and growth rates during autumn. The likely functional advantage of haplotype No05 emerged because eye infections by *Diplostomum* sp. impair vision, affecting foraging behaviour. Conversely, resistance to the nematode *Camallanus lacustris* was found for individuals with haplotype No36.No54, but only in spring. Combining parasite diversity and MHC haplotypes in a co-inertia analysis, we found that MHC-parasite associations are strongest when parasite species are most diverse and abundant, i.e. when parasite-mediated selection is strongest. Overall, our results suggest that differential resistance values over seasonal variation results in differential selection on MHC haplotypes. If this contributes to differential mating success, this has implications for the maintenance of MHC polymorphism.

## Introduction

Seasonal change is a pervasive source of environmental variation (Fretwell 1972; Boyce 1979; Altizer et al. 2006). Determining its ecological and evolutionary consequences, however, remains challenging, in part because seasonal change reflects many simultaneous biotic and abiotic changes (Altizer et al. 2006; Troost et al. 2009). Parasite-mediated selection varies seasonally (e.g. Dunsmore and Dubzinski 1968; Hanek and Fernando 1978; Dowell 2001) and has important implications for population and evolutionary dynamics (Poulin 2007): Parasitic nematodes, for instance, regulate population cycles in the red grouse (*Lagopus lagopus scoticus*, Hudson et al. 1998), and differences in parasite community drive local adaptation in river and lake populations of three-spined sticklebacks (*Gasterosteus aculeatus*, Eizaguirre et al. 2012*b*). Yet, how seasonal variation affects host fitness and the evolution of host resistance is largely unexplored (Schrader et al. 2003).

Variation in parasite communities and loads across seasons can be linked to four dominant factor (Alitzer et al. 2006). First, the abundance and development of infective life stages often vary with abiotic conditions, as seen in larvae of the sheep helminth *Ostertagia ostertagi*, where warm and humid weather favours their development (Stromberg 1997). Second, seasonal host aggregations or social behaviour can favour transmission of many directly infecting parasites, as found in bats infected by ectoparasitic mites (Van Schaik and Kerth 2017). Third, host immunity may also change as a by-product of altered abiotic conditions such as temperature, resulting in seasonal expression patterns of genes involved in immunity (Stewart et al. 2018). And lastly, coinfection by several parasites can alter host-parasite interactions, as observed for three-spined stickleback infected with *Schistocephalus solidus*, experiencing increased coinfection with the eye fluke *Diplostomum pseudospathaceum* (Benesh and Kalbe 2016).

In vertebrates, the genes of the Major Histocompatibility Complex (MHC) are central to the adaptive immunity (Janeway et al. 2005). This highly polymorphic, gene dense region encodes for immunologically important cell surface proteins. Proteins encoded by MHC Class II$\beta$ genes, in

particular, present peptides from parasite-derived antigens at the cell surface to be recognised by T cells. Foreign antigens will then trigger a highly parasite-specific immune reaction through the activation of T cells (Janeway et al. 2005). As a consequence of differential binding-affinities, distinct MHC alleles determine an individual's parasite-specific resistance and hence, fitness (Bernatchez and Landry 2003). Negative frequency-dependent selection, heterozygote advantage and habitat heterogeneity combined contribute to local adaptation and maintain an exceptionally high allelic diversity within a population (Eizaguirre and Lenz 2010; Spurgin and Richardson 2010; Eizaguirre et al. 2012*a*). A less understood component of habitat heterogeneity is its temporal element. For instance, the frequency of the MHC Class II*β* allele Pore_a132 in Trinidadean guppies (*Poecilia reticulata*) changes between years following the infection rate with the monogenean *Gyrodactylus* sp. (Fraser et al. 2010). This implies that, similarly to spatial differences, temporal mismatches between functional MHC alleles and parasite diversity and abundance may lead to fluctuating selection across seasons.

The three-spined stickleback (*Gasterosteus aculeatus*) is a model species for studying host-parasite coevolution (Barber 2013; Stewart et al. 2017). Annual and seasonal fluctuations of parasite load and diversity have been recorded in a variety of populations (e.g. Chappell 1969; Pennycuick 1971; Kalbe et al. 2002; MacColl 2009; Eizaguirre et al. 2011; Young and MacColl 2017). Additionally, the class II*β* regions of the MHC are well characterised for this species (Lenz et al. 2009*a*). Both individual MHC diversity and specific MHC alleles provide resistance and impact host condition and reproductive fitness (Wegner et al. 2003*a*; Eizaguirre et al. 2009*b*; Kalbe et al. 2009; Lenz et al. 2009*b*). Differential fitness owing to specific MHC alleles shapes the change in MHC frequency across generations and patterns of local adaptation between hosts and parasites (Eizaguirre et al. 2012*b*, 2012*a*).

We aimed here to address how seasonality shapes host-parasite interactions by exposing laboratory-bred sticklebacks with different MHC genotypes to their natural parasite fauna in their lake of origin

at three distinct time points (spring, summer, and autumn). For each season, we estimated survival, and determined body condition, growth, and parasite load in order to document changes in the functional advantages of MHC alleles across seasons. We predict variability in the specific MHC-parasite associations between seasons. This may result in differential fitness associated with resistance. Throughout the seasons, we expect the associations between parasite community and host MHC diversity to be strongest when parasite-mediated selection is most intense.

**Methods**

*FISH COLLECTION AND BREEDING*. In August 2016, three-spined stickleback (*G. aculeatus*) were collected with minnow traps from the Großer Plöner See (54°9'21.16'N, 10°25'50.14'E, Germany). These fish were used to breed *in vitro* 13 independent families of $F_1$ offspring. The egg clutches were maintained in 1 L glass jars at 18°C for up to 8 days until hatching. Water was exchanged daily. The hatched fry was kept in 16 L tanks separated by families for 6-8 weeks. At 2 months of age, the families were split into groups of 20 fish and reared on frozen chironomid larvae 5 days per week. Prior to the experiment, fish were brought through autumn (12°C; 12hr day length (DL); 3 weeks), winter (6°C; 8hr DL; 6 weeks) and spring (12°C; 12hr DL; 3 weeks) conditions. Upon change to summer conditions (18°C; 16hr DL), fish were fed with live prey *ad libitum* (Chironomid larvae; *Artemia salina*) before the experiment commenced 3 weeks later.

*TRANSPLANT EXPERIMENT*. Prior to transplant 20 fish from each family were measured, weighed and spine-clipped. Two random fish per family for each family were sorted into ten 16 L tanks, yielding 26 fish per tank. The fish were then released into their cages in different season (spring: 29.05.2017; summer: 31.07.2017; autumn: 02.10.2017). Due to mortality, not all families had enough individuals for the final transplant experiment in October, but the density in the tanks was maintained by adding additional individuals from other families. Each group of fish was then acclimatized to the lake water temperature (spring: 15.8°C; summer 19.1°C; autumn 14.3°C) by progressively adding lake water into their holding tanks over two hours. After this, each group was transferred to one of

ten stainless steel cages (Length: 100 cm; width: 60 cm; Height 25 cm; Volume: 1.5 $m^3$, Eizaguirre et al. 2012*b*), placed 15 m offshore at 1-1.5 m depth, 50 cm from each other. Allowing 5 weeks of parasite exposure, we then recovered two cages per day for 5 consecutive days. All recovered fish were immediately measured, weighed and screened for ecto- and endo-parasites following Kalbe et al (2002). Finally, the dorsal fin was clipped for DNA extraction and re-identification.

*MICROSATELLITE AND MHC GENOTYPING*. DNA extractions from spines and fins were performed using DNeasy 96 Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. All fish were genotyped for nine microsatellites combined in two different multiplexed PCRs using primers optimised for this population (Kalbe et al. 2009). Microsatellite information was then used to re-identify fish post experiment. The MHC class II*β* genotypes of all fish was determined using reference-strand-mediated conformation analysis optimised for three-spined sticklebacks (RSCA, Lenz et al. 2009*a*). We targeted the exon II of the MHC class II genes which encodes the highly variable peptide-binding beta chain region of the final molecule. Notably, the MHC II*β* genes in stickleback are duplicated and frequently occur in tightly linked alleles, which we refer to as haplotypes (Lenz et al. 2009*a*). Based on these results, we assigned MHC genotype and haplotypes as well as determined number of MHC alleles per individual.

*PARASITE AND CONDITION INDICES*. Individual parasite number and diversity were combined into an 'Individual Parasite Index' ($I_{PI}$, Kalbe et al. 2002). Fish weight and length from before and after the experiment were used to calculate initial and final body conditions following $(weight/length)^b x100$ with *b* being standardised at 3.00 (Frischknecht 1993). Gonad weights were subtracted from body weight to compute final body condition. Growth rate was determined as (final length – initial length)/time spent in the cages.

*DATA ANALYSES*. All statistical analyses and graphical visualizations were performed with R version 3.3.1 (R Core Team 2016; packages include: '*plyr*', '*vegan*', '*reshape2*', '*ggplot2*',

'*lmerTest*', '*lme4*', *'ade4'*). Model residuals were tested for normality and homoscedasticity of variance. Data were transformed if required to meet test assumptions. All models were backward-selected using the *anova* function.

*PARASITE LOAD AND COMMUNITY.* To determine whether there are differences in parasite richness or load ($I_{PI}$; log transformed) across seasons, we performed two mixed effect model with season and sex as fixed factors and family and cage as random effects. To identify the main source of variation in parasite load we also ran a component variance analysis using a linear mixed effect model season, MHC genotype, cage and family as factors. Furthermore, to determine whether there were different parasite communities across seasons, we performed a PERMANOVA on a Bray-Curtis dissimilarity matrix with cage as block factor, and log-transformed parasite species' abundance. Next, we ran a similarity percentage analysis (SIMPER) to isolate pairwise differences in parasites between subsequent seasons. Lastly, we tested for signs of coinfection using a multi-correlational approach between all parasite species' abundance and using a Bonferroni correction to account for multiple testing.

*LINKING HOST MHC AND MACROPARASITE DIVERSITY.* In order to identify which MHC haplotypes associated with resistance to specific parasite species, we split parasite species identified as seasonally variable by the previous SIMPER into three equal groups (high, intermediate and low infection load) for each season. We then ran a PERMANOVA with MHC haplotype matrix as response and parasite species infection grouping (i.e. low vs high) as an explanatory variable and cage as a block factor. Since the MHC haplotype matrix is a presence/absence matrix, we used Jaccard distance. This was followed by a new SIMPER analysis in case of significant PERMANOVA results. Subsequently, we ran several mixed effect models. Each model was run within the season where the effect was discovered by the SIMPER and contained the identified MHC haplotype, initial body condition, MHC zygosity and their interactions as explanatory variables. Family and cage were kept as random factors. We used 'false discovery rate' correction in case of multiple testing (shown as

$p_{fdr}$). Retained significant results for specific MHC haplotypes led to test the relationship of those MHC haplotypes associated with resistance in each of the other seasons to understand whether and how the relationship changes.

To gain a broader overview of the association between fish MHC haplotypes and parasite diversity, we performed a co-inertia analysis (Doledec and Chessel 1994). Separated by season, we ran a correspondence analysis on MHC haplotype diversity. Then, we computed a principle component analysis on the parasite species abundances after weighting each row according to the correspondence analysis. From there, we asked whether the observed coefficient of correlation between the two hyperspaces – lies outside the 95% confidence interval of 100 random co-inertia analyses. The coefficient varies between 0 and 1, with values closer to 1 indicating a stronger correlation between the MHC and parasite datasets.

*FITNESS CONSEQUENCES*. While resistance towards parasites is already an important fitness advantage, MHC associated-resistance should coincide with additional measurable fitness effects, such as increased body condition and/or growth. Both of those measurements were used as response variables in two linear mixed effect models with season, sex, $I_{PI}$, MHC haplotype zygosity, specific haplotypes and their interactions as explanatory variables and family and cage as random effects. We also assessed survival using a generalised mixed effect model with Laplace approximation with season, initial body condition, specific MHC haplotype and their interactions as explanatory variables and family and cage as random effects.

**Results**

*PARASITE LOAD AND COMMUNITY*. Out of a total of 16 parasite species identified, 13 were recorded in spring, 14 in summer and 9 in autumn. Mean individual parasite richness differed among seasons (spring: 4.5 (±0.1SE=standard error); summer: 4.9 (±0.1SE); autumn: 1.6 (±0.1SE); $F_{2,674}$=547.85, $p<0.001$). Parasite load also differed among seasons with fish in summer having the

highest parasite load ($F_{2,674}$=520.00, p<0.001). No differences between sexes was observed in individual parasite richness ($F_{1,676}$=0.06, p=0.814) or load ($F_{1,676}$=0.47, p=0.495). The highest variance in parasite load was explained by season 49% whereas MHC genotype and family both explained roughly 5% (Supplementary Table 1). The parasite community also differed significantly among seasons ($F_{2,669}$=443.7, p<0.001; Figure 1A). The SIMPER analysis revealed those parasite species that significantly explain the difference among seasons (Supplementary Table 2; Figure 1B). Infection with the eye fluke *Diplostomum* sp., while always present, was highest over summer. In contrast, Glochidia, the parasitic larvae of some freshwater bivalves, was only found in June, but at high intensity, while *Gyrodactylus* sp. increased in frequency and was most abundant in autumn. Other parasites, such as *Camallanus lacustris,* occurred at comparably low abundance throughout the seasons. In terms of coinfection, *Diplostomum* sp. and Glochidia loads were negatively correlated after Bonferroni correction (*R*=-0.53, p<0.001; Supplementary Figure 1). *Diplostomum* sp. infections also correlated positively with both the number of *Apatemon gracilis* (*R*=0.52, p<0.001) and the trophically transmitted nematode *C. lacustris* (*R*=0.49, p<0.001; Supplementary Figure 1).

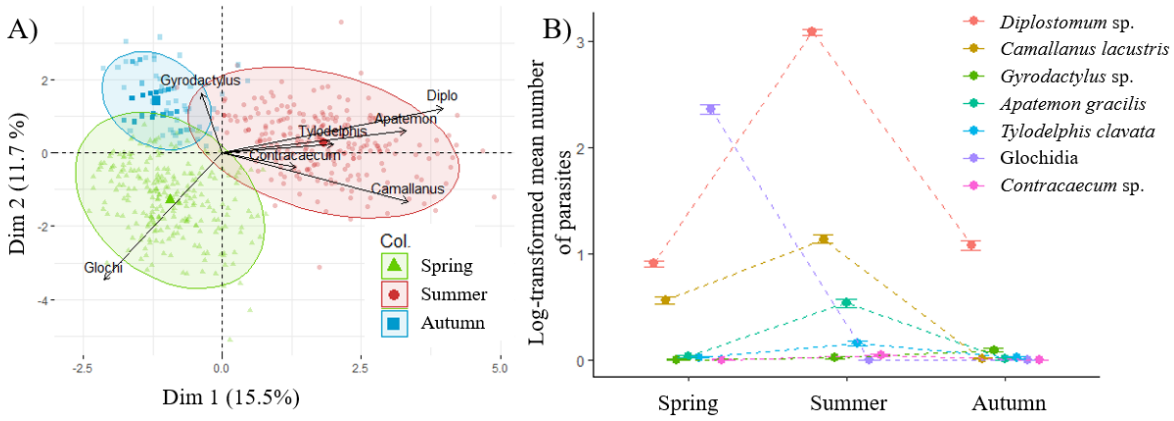


**Figure 1 A) PCA showing parasite community across seasons (green=spring, red=summer, autumn=blue); B) seasonal changes in abundances (mean number ±SE; log-transformed) of the common and seasonally variable parasites identified in this study (Supplementary Table 2).**

*LINKING HOST MHC AND MACROPARASITE DIVERSITY*. Using PERMANOVAs (Supplementary Table 3) and SIMPER (Supplementary Table 4), we identified several MHC haplotypes associated with high and low load of *Diplostomum* sp. in spring, summer and autumn, and confirmed the results using mixed-effect models (Supplementary Table 5): Individuals with haplotype No05 (allele No05, NCBI accession number AY687829) showed increased resistance towards *Diplostomum* sp. in all three seasons (All $p_{fdr} < 0.05$; Table 1; Figure 2A). MHC haplotype No39.No40.No41 (alleles No39 see NCBI accession number AAY34959; No40 and No41 see Appendix 1) on the other hand, was associated with resistance to *Diplostomum* sp. in spring ($F_{1,89}=6.33$, $p_{fdr}=0.19$) and autumn ($F_{1,185}=5.77$, $p_{fdr}=0.056$), but only showed weak association in summer ($F_{1,217}=3.35$, $p=0.207$, Supplementary Table 4, 5). Noteworthy, because this haplotype only occurred in one fish family, we excluded it from further analyses. Haplotype No15.No16 (alleles No15 and No16, NCBI accession number DQ016410 and DQ015617, respectively) was associated with increased susceptibility to *Diplostomum* sp. in spring only ($F_{1,92}=7.58$, $p_{fdr}=0.014$; Figure 2B). In summer, the model also revealed that initial body condition was an important correlate of infection load with *Diplostomum* sp. (Table 1).

**Table 1. Summary table showing the effect of sex, initial body condition, specific MHC haplotype and haplotype zygosity on parasite infection load with *Diplostomum* sp. across three different seasons;** All models were backward selected using the *anova* function in R; Significant results are highlighted in bold.; d.f. denotes degrees of freedom.

| Season | explanatory variables | d.f. | F-value | p-value |
|---|---|---|---|---|
| Spring | **MHC haplotype No05** | **1, 243** | **18.197** | **<0.001** |
| | Haplotype Zygosity | 1, 241 | 1.372 | 0.243 |
| | Initial Body Condition | 1, 242 | 0.814 | 0.368 |
| | Sex | 1, 245 | 0.336 | 0.563 |
| Summer | **MHC haplotype No05** | **1,235** | **19.437** | **<0.001** |
| | Haplotype Zygosity | 1,235 | 0.092 | 0.761 |
| | **Initial Body Condition** | **1,234** | **11.287** | **0.001** |
| | Sex | 1,228 | 0.105 | 0.746 |
| Autumn | **MHC haplotype No05** | **1,38** | **5.256** | **0.028** |
| | Haplotype Zygosity | 1,47 | 0.009 | 0.926 |
| | Initial Body Condition | 1,132 | 3.169 | 0.077 |
| | Sex | 1,174 | 0.052 | 0.820 |

Following the same analytical framework for infections with *Camallanus lacustris* (Supplementary Table 3-5), we identified No36.No54 (alleles No36 see NCBI accession number DQ016411; No54 see Appendix 1) to be linked to increased resistance in spring ($F_{1,60}$=7.11, $p_{fdr}$=0.030, Supplementary Figure 2A) but not in summer ($F_{1,147}$=0.553, p=0.458) and autumn ($F_{1,192}$=0.047, p=0.829).

Lastly, we identified the haplotype No08.SCX15 (alleles No08 and SCX15, NCBI accession number AY687842 and EU541449, respectively) to be associated with higher susceptibility to *Gyrodactylus* sp. in summer ($F_{1,238}$=17.38, p<0.001) and autumn ($F_{1,43}$=6.86, $p_{fdr}$=0.024, but not spring ($F_{1,249}$=0.19, p=0.657, Supplementary Figure 2B; Supplementary Table 5).

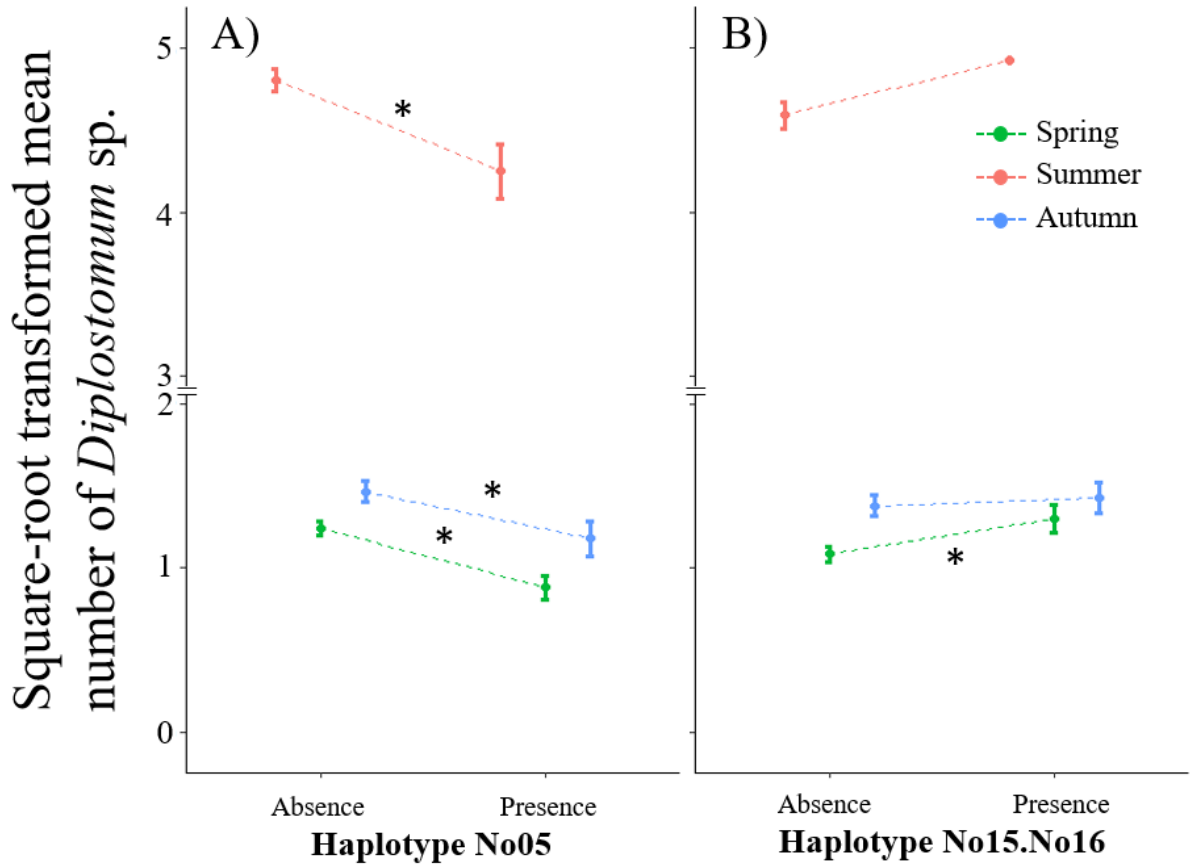


**Figure 2. Infection load with *Diplostomum* sp. (mean ±SE; square-root transformed) in the presence or absence of MHC A) Haplotype No05, and B) No15.No16 in spring (green), summer (red) and autumn (blue).**

Using a co-inertia analysis, we further show that in summer, when parasite diversity and load are highest, MHC haplotypes and parasite diversity were strongly correlated (Fig 3B; RV-Coef.: 0.051, p=0.01). Similarly, MHC haplotypes and parasite diversity were correlated in autumn, but the relationship was much weaker than in the previous seasons (Fig 3C; RV-Coef.: 0.027; p=0.02). This suggests that the ecological relevance of MHC is at its highest when parasite selection is most severe.

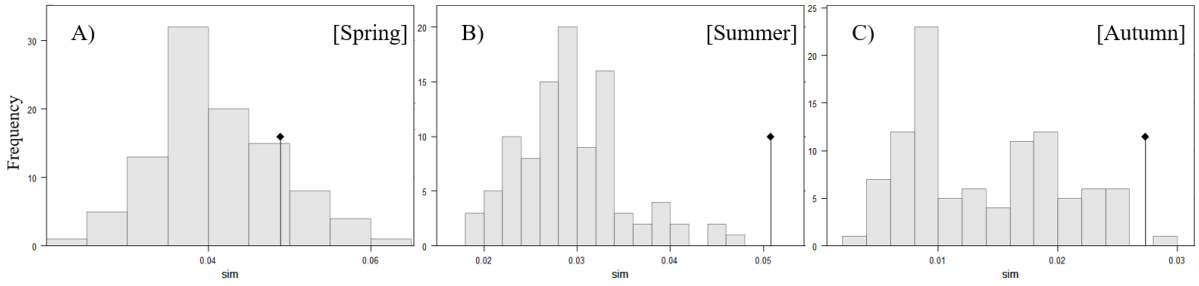


**Figure 3. Frequency distribution of the first axis $R^2$ value for 100 random co-inertia analyses and the RV value observed in spring, summer and autumn indicating similarity between data sets.**

*FITNESS CONSEQUENCES*. Fish from the spring experiment had higher overall body condition than those from the autumn experiment (Table 2a; e.g. $F_{2,661}$=317.82, p<0.001). An interaction between sex and season on final body condition revealed that females were in lower body condition than males (e.g. $F_{2,666}$=8.03; p<0.001; Tukey-test: spring/♀ vs. spring/♂ , p<0.001; summer/♀vs. summer/♂, p<0.001; autumn /♀vs. autumn/♂, p=0.018). Moreover, an interaction between seasons and parasite load showed that body condition declined in summer more strongly with increased parasite load than in the other seasons (e.g. $F_{2,671}$=4.11, p=0.017; Figure 4A), indicating that the strength of parasite-mediated selection has season-dependent negative fitness consequences. Individuals with MHC haplotype No05 had also higher body condition ($F_{1,553}$=4.12, p=0.043, $p_{fdr}$=0.172; Figure 4B).

**Table 2. Summary table of linear mixed effect models assessing the effect of season, sex, parasite load, specific MHC haplotypes and MHC haplotype zygosity on a) final condition and b) growth;** All models were backward selected using the *anova* function in R; Significant results are highlighted in bold.; d.f. denotes degrees of freedom.

| | a) Condition | | | b) Growth | | |
|---|---|---|---|---|---|---|
| ***Explanatory variables*** | ***d.f.*** | **F-value** | **p-value** | ***d.f.*** | **F-value** | **p-value** |
| **Season** | 2,661 | 407.51 | **<0.001** | 2,666 | 595.95 | **<0.001** |
| **Sex** | 1,664 | 142.14 | **<0.001** | 1,674 | 0.84 | 0.361 |
| **Haplotype No05** | 1,553 | 4.12 | **0.043** | 1,171 | 0.13 | 0.722 |
| **Haplotype Zygosity** | 1,470 | 2.07 | 0.151 | 1,109 | 0.11 | 0.741 |
| **Parasite load corrected for season** | 1,669 | 13.73 | **<0.001** | 1,675 | 38.06 | **<0.001** |
| **Season * Haplotype No05** | - dropped - | | | 2,675 | 5.41 | **0.005** |
| **Season * sex** | 2,666 | 8.03 | **<0.001** | 2,679 | 20.13 | **<0.001** |
| **Season * Parasite load corrected for season** | 2,671 | 4.11 | **0.017** | 2,673 | 7.65 | **<0.001** |
| **Season** | 2,660 | 408.02 | **<0.001** | 2,665 | 838.38 | **<0.001** |
| **Sex** | 1,663 | 139.73 | **<0.001** | 1,674 | 1.09 | 0.298 |
| **Haplotype No36.No56** | 1,537 | 0.05 | 0.825 | 1,158 | 1.61 | 0.206 |
| **Haplotype Zygosity** | 1,509 | 0.76 | 0.383 | 1,121 | 0.46 | 0.500 |
| **Parasite load corrected for season** | 1,669 | 15.56 | **<0.001** | 1,677 | 37.33 | **<0.001** |
| **Season * sex** | 2,665 | 8.72 | **<0.001** | 2,673 | 7.34 | **<0.001** |
| **Season * Parasite load corrected for season** | 2,669 | 4.40 | **0.013** | 2,678 | 18.06 | **<0.001** |
| **Season** | 2,661 | 408.47 | **<0.001** | 2,654 | 194.25 | **<0.001** |
| **Sex** | 1,662 | 139.71 | **<0.001** | 1,656 | 39.64 | **<0.001** |
| **Haplotype No15.No16** | 1,602 | 0.32 | 0.572 | 1,544 | 2.30 | 0.130 |
| **Haplotype Zygosity** | 1,527 | 0.84 | 0.360 | 1,431 | 0.34 | 0.558 |
| **Parasite load corrected for season** | 1,669 | 15.56 | **<0.001** | 1,665 | 7.06 | **0.008** |
| **Season * Haplotype No15.No16** | - dropped - | | | 2,656 | 4.60 | **0.010** |
| **Season * sex** | 2,665 | 8.78 | **<0.001** | - dropped - | | |
| **Season * Parasite load corrected for season** | 2,670 | 4.42 | **0.012** | - dropped - | | |
| **Season** | 2,660 | 407.47 | **<0.001** | 2,666 | 843.55 | **<0.001** |
| **Sex** | 1,662 | 140.08 | **<0.001** | 1,102 | 0.68 | 0.412 |
| **Haplotype No08.SCX15** | 1,404 | 0.73 | 0.393 | 1,673 | 1.00 | 0.319 |
| **Haplotype Zygosity** | 1,475 | 0.61 | 0.435 | 1,678 | 38.18 | **<0.001** |
| **Parasite load corrected for season** | 1,670 | 15.09 | **<0.001** | 1,134 | 0.39 | 0.535 |
| **Season * sex** | 2,666 | 8.44 | **<0.001** | 2,673 | 7.31 | **<0.001** |
| **Season * Parasite load corrected for season** | 2,670 | 4.36 | **0.013** | 2,678 | 17.68 | **<0.001** |

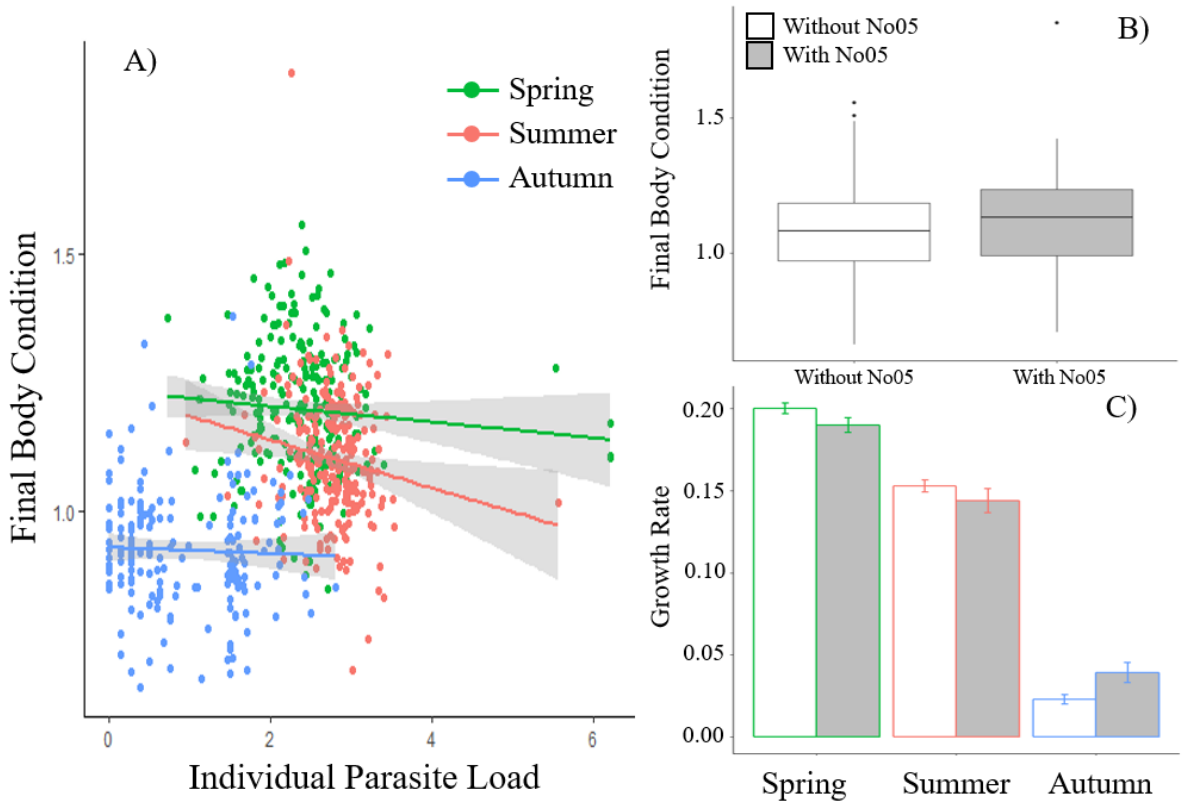


**Figure 4. A) Relationship between parasite load (log-transformed) and host final body condition in different seasons (green=spring, red=summer, blue=autumn). B) Body condition and C) growth (mean ±SE) in relation to presence (grey) and absence (white) of the MHC haplotype No05 and across seasons (for colours see above).**

Similarly to body condition, growth rate was highest during spring and lowest in autumn (Table 2b; e.g. $F_{1,666}$=595.95, p<0.001). An interaction between sex and season showed, however, that only in spring females grew more than males ($F_{2,673}$=7.65, p<0.001; Tukey-test: spring/♀ vs. spring/♂, p<0.008; others – p=ns). In addition, a season by parasite load interaction revealed that parasite load was negatively associated with growth, particularly during summer ($F_{2,675}$=20.13, p<0.001), strengthening the argument of seasonally variable parasite-mediated selection. Moreover, an interaction between the presence of haplotype No05 and the season of exposure showed that

individuals with this haplotype experienced increased growth, mainly in autumn (Figure 4C, $F_{2,675}$=5.41, p=0.005; Tukey test: autumn/No05- vs. autumn/No05+, p<0.05; others – p=ns). Lastly, an interaction between haplotype No15.No16 and season suggests reduced growth in summer, while fish with this haplotype grew more in spring and autumn ($F_{2,656}$=4.60, p=0.010; Tukey-test: p=ns).

Fish survival varied amongst seasons ($F_{2,736}$=6.05, p=0.002; spring: 254, summer: 247, autumn: 218 out of 260 fish; Table 3). Interestingly, we identified an interaction between initial body condition and season: high initial body condition in spring meant a lower chance of survival, whereas high initial body condition in autumn increased survival chances ($F_{2,736}$=4.82, p=0.008; Supplementary Figure 3). Furthermore, individuals with haplotype No05 survived less ($F_{1,736}$=11.21, p<0.001, $p_{fdr}$=0.004), while those individuals with the susceptibility-associated MHC haplotype No08.SCX15 ($F_{1,736}$=4.27, p=0.039, $p_{fdr}$=0.052) and No15.No16 ($F_{1,736}$=5.54, p=0.019, $p_{fdr}$=0.038) survived more, suggesting life history trade-offs between resistance and longevity.

**Table 3. Summary table of generalised linear models using season, initial body condition and specific MHC haplotype to explain differential survival;** All models were backward selected using the *anova* function in R; Significant results are highlighted in bold.; d.f. denotes degrees of freedom.

| | **Survival** | | |
|---|---|---|---|
| ***Explanatory Variables*** | ***d.f.*** | **F-value** | **p-value** |
| **Season** | 2,736 | 6.05 | **0.002** |
| **Haplotype No05** | 1,736 | 11.21 | **<0.001** |
| **Initial Body Condition** | 1,736 | 0.18 | 0.672 |
| **Season * Initial Body Condition** | 2,736 | 4.82 | **0.008** |
| **Season** | 2,736 | 7.01 | **0.001** |
| **Haplotype No36.No54** | 1,736 | 0.53 | 0.468 |
| **Initial Body Condition** | 1,736 | 0.01 | 0.927 |
| **Season * Initial Body Condition** | 2,736 | 5.26 | **0.005** |
| **Season** | 2,736 | 5.97 | **0.003** |
| **Haplotype No15.No16** | 1,736 | 5.54 | **0.019** |
| **Initial Body Condition** | 1,736 | 0.00 | 0.977 |
| **Season * Initial Body Condition** | 2,736 | 4.46 | **0.012** |
| **Season** | 2,736 | 7.02 | **0.001** |
| **Haplotype No08.SCX15** | 1,736 | 4.27 | **0.039** |
| **Initial Body Condition** | 1,736 | 0.08 | 0.777 |
| **Season * Initial Body Condition** | 2,736 | 5.20 | **0.006** |

**Discussion**

Seasonal variation in parasite-mediated selection is likely an important temporal selection pressure on hosts (Altizer et al. 2006). Yet, the effect of such variation have not been addressed for MHC-based resistance and fitness. Here, we showed that parasite community and infection intensity vary throughout the year. Individual parasite burden and richness are highest in summer. We also show MHC-associated advantages vary throughout the season: MHC haplotype No05, for instance, provided resistance against *Diplostomum* sp. – a particularly prevalent eye fluke – with positive consequences for body condition and growth but reduced survival. At the same time, MHC haplotype No36.54 provided resistance to *C. lacustris* in spring only showing the temporal variation of MHC associated resistance. Lastly, using a co-inertia analysis, we showed that the correlation between

parasite community and MHC-haplotype pool is highest when parasite-mediated selection is strongest. These findings suggest a context-dependent association between parasites and MHC-based resistance across seasons, with possible implications for the maintenance of MHC polymorphism.

As abiotic conditions, such as temperatures or precipitations, change along a seasonal gradient, parasite diversity and abundance also changed (Altizer et al. 2006). Parasite-mediated selection was strongest during summer when we recorded the highest parasite diversity and burden. However, we also documented parasite-specific seasonal variation that illustrate how abiotic conditions, condition-dependent host immunity, and parasite life histories impact parasite communities across seasons (Altizer et al. 2006): Glochidia, for instance, was only present early in the season in line with the bivalves' life history (Watters and O'Dee 2000). *Gyrodactylus* sp. was most prevalent during autumn. This could be due to changes in host immunocompetence (Stewart et al. 2018) or in population growth rate of *Gyrodactylus* sp. as temperatures drop (Andersen and Buchmann 2009). The eye fluke *Diplostomum* sp. was common throughout all seasons but most prevalent during August possibly owing to the abundance of its intermediate snail host during summer (Chappell 1969; Pennycuick 1971; Kalbe et al. 2002).

Interestingly, *Diplostomum* sp. burden was correlated with infection by the eye fluke *Apatemon gracilis* and the actively manipulating gastrointestinal nematode, *C. lacurstris*. Such interspecific associations between parasite species can emerge due to direct competition (Knowles et al. 2013), covariance in transmission rate (Lotz et al. 1995) or exploitation of host manipulation by other parasites (Thomas et al. 1998). In fact, both eye flukes were found more frequently in hosts infected with the immunosuppressant helminth *S. solidus* in past experiments (Benesh and Kalbe 2016). And since *C. lacustris* is also known to manipulate its copepod intermediate hosts (Hafer and Milinski 2016), this might have potentially facilitating coinfection with both actively infecting flukes as seen in our case. At the same time, shared life histories resulting in the infection of the fish eyes, as well as transmission pathways, may have promoted coinfection of the two trematodes.

We also showed a link between the MHC haplotype No05 and resistance to *Diplostomum* sp. throughout the year. This parasite deteriorates host vision and, consequently, affects foraging efficiency (Crowden and Broom 1980). Resistance was associated with increased body condition across all seasons and growth in autumn, highlighting the extended fitness benefits of lower parasite load. On the other hand, haplotype No36.No54 was associated with resistance to *C. lacustris* in spring but without detectable fitness impacts. Furthermore, fish with haplotype No15.No16 showed evidence of susceptibility to *Diplostomum* sp. in spring, while those with haplotype No08.SCX15 displayed susceptibility to *Gyrodactylus* sp. in summer and autumn. Neither showed declining fitness though. Together however, these outcomes underscore the context-dependent nature of MHC-based resistance in a time-related manner compared to that of spatial heterogeneity (Hedrick 2002).

Interestingly, fish with haplotypes associated with resistance experienced higher mortality than those with haplotypes linked to susceptibility. Such life history trade-offs are not rare (e.g. Hanssen et al. 2004; Graham et al. 2010): Greater Sac-Winged Bats (*Saccopteryx bilineata*), for instance, trade longevity for immunocompetence as bats with high immunoglobulin G concentrations at capture were less likely to survive the next six months(Schneeberger et al. 2014). This is in line with our finding where fish with MHC haplotype No05 resist *Diplostomum* sp., are in better condition and grow faster, but die earlier. By contrast, overwintering survival of yearlings in Soay sheep (*Ovis aries*) was linked to three MHC class II alleles owing to their role in resistance against intestinal nematodes (Paterson et al. 1998). Such a positive relationship where resistance is associated with increased fitness and survival may have existed at an earlier life stage than that of our experimental fish. This is because juvenile fish with haplotype No05 could have benefited from higher growth and body condition by increasing overwintering survival (Francova and Ondrackova 2013). The context-dependence of MHC associated resistance may therefore extend beyond a seasonal gradient and be linked with organism's life stage.

Combined, these findings highlight the importance of studying gene-by-environment interactions across time. Many studies on MHC-parasite associations focus on a single time point (e.g. Paterson et al. 1998) and few track these associations through time (e.g. Fraser et al. 2010). But we know that parasite-mediated selection varies with time. For example, the prevalence of *Gyrodactylus* sp. declined dramatically between years and caused alleles formerly associated with resistance to lose their functional MHC associated advantage in earlier studies (Eizaguirre et al. 2009*b*; Lenz et al. 2009*b*). In the present study, we show a rapid turnover in parasite diversity and that different strengths of parasite-mediated selection across seasons likely cause temporal mismatches with host immune genes. These links remain, however, strongest when parasite-mediated selection itself was strong as demonstrated by the results from the co-inertia analysis. But this also implies that under weaker parasite-mediated selection other traits than the MHC might be important, such as body size which determines anti-predator behaviour in stickleback (Külling and Milinski 1992). This illustrates the multifaceted nature of selection along the life time of an individual host.

To sum-up, temporal heterogeneity in parasite-mediated selection is an essential evolutionary pressure on hosts. With regards to the MHC, seasonal variation in parasite communities may impose fluctuating selection on host populations in a context-dependent manner, similar to that detectable over spatial scales. We therefore suggest a temporal mosaic of selection pressures across seasons. Such differential selection particularly before reproduction and during mating periods may have ramifications for the maintenance of MHC polymorphism within populations (Eizaguirre et al 2009, Chapter 2).

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# CHAPTER 6. VARIATION AT IMMUNO-GENES PREDICTABLY CHANGES FEMALE MATE CHOICE UNDER SEMI-NATURAL CONDITIONS

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**Subject Areas:** host-parasite interactions

**Keywords:** Major Histocompatibility Complex, host-parasite interactions, sexual selection, *Gasterosteus aculeatus*, indirect genetic benefits, mate choice, good genes.

**Abstract**

Mate choice for indirect genetic benefits is common in nature. The best known genetic basis for mate choice are genes involved in parasite resistance, the genes of the Major Histocompatibility Complex (MHC). Both MHC-based mate choice for good genes and compatibility are well studied and may not be mutually exclusive. Here, we tested the hypothesis that the presence of fish carrying good genes, in the form of known resistance MHC alleles, should result in mate choice for them leading to stronger directional sexual selection than observed in populations where fish carrying those alleles have been removed. Focusing on three-spined stickleback, we first identified an association between a MHC haplotype and resistance to the eye fluke *Diplostomum* sp. in a field cage experiment. Then, we assembled replicated populations with and without this resistance MHC haplotype and allowed natural reproduction and parasite infection to take place in semi-natural enclosures. In the presence of the resistance MHC haplotype, females chose males carrying this haplotype over genetically compatible mates. In absence of males with this resistance haplotype, females chose based on MHC compatibility. On the other hand, males engaged more frequently in sneaking behaviours in the absence of the resistance haplotype. Overall we show stronger sexual selection via mate choice in the presence of resistance MHC haplotypes. We suggest that mate choice based on good genes functions similar to parasite-mediated negative frequency dependent selection, leading to a rapid depletion of its additive genetic benefit at high frequency and ultimately, to selection for compatible genes.

## Introduction

Mate choice is an important evolutionary process since it aids in local adaptation, drives speciation and protects against extinction (Andersson 1994; Milinski 2014; Lumley et al. 2015). Mate choices are typically based on direct benefits, such as the provision of food or nests (Møller and Jennions 2001), or for extravagant ornaments (Hamilton and Zuk 1982; Kokko et al. 2003). The latter, in particular, indicate a mates genetic quality (Tregenza and Wedell 2000; Mays and Hill 2004), such as beak colour in blackbirds (*Turdus merula*, Faivre et al. 2003) or odour in three-spined stickleback (*Gasterosteus aculeatus,* (Milinski et al. 2010).

Mate choice for such indirect genetic benefits emerges from two non-mutually exclusive mechanisms, selection for good genes and/or for compatibility (Mays and Hill 2004). Mate choice for good genes stems from selection for condition-dependent traits that honestly signal a mates' genetic quality, such as plumage colour in passerines (Hamilton and Zuk 1982) or nuptial colouration in fish (Milinski and Bakker 1990). As such, good genes increase an individual's reproductive fitness as well as increase variance in reproductive success between sexes within a population (Andersson 1994). The benefits of a choice for compatible genes, on the other hand, are non-additive and arrise from the combination of parental genotypes producing offspring of theoretically high quality (Tregenza and Wedell 2000; Mays and Hill 2004). Because compatibility is based on self-reference every choice is relative to the choosy individual's genetic make-up (Aeschlimann et al. 2003; Milinski et al. 2005), leading to little reproductive variance between the sexes (Milinski 2006). Overall, while both theories posit increased offspring quality, predictions can be made at the parental generation with differences in variation of reproductive successes between sexes and between individual carrying the good genes and those lacking it.

Choices for good and for compatible genes are context-dependent (Neff and Pitcher 2005). For example, as male density increases and operational sex ratio changes, choosiness, and as such, strength of sexual selection, increases (Emlen and Oring 1977; Eizaguirre et al. 2009*b*). Alternatively,

concomitant selection can weaken female preference - and, hence, sexual selection for good genes - as seen in female guppies (*Poecilia reticulata*) which adjust their preference for male display colours according to the level of predation risk they experience (Houde and Endler 1990, Chapter 3, 4). Differences in choice for good genes or compatible genes may also emerge from genetic variation between or within populations (Colegrave et al. 2002; Neff and Pitcher 2005). This is because when additive genetic variation in a population is large, selection based on good genes is advantageous and leads to directional selection (Pitcher and Neff 2006). By contrast choice for compatibility may emerge from high non-additive genetic variation or the lack of additive genetic benefits (Neff and Pitcher 2005). But in comparison to density-mediated effects or antagonistic selection, experimental evidence for how a population's genetic make-up influences female mate choice and alters the strength of sexual selection is outstanding.

In jawed vertebrates the best known genetic basis of mate choice are the genes of the Major Histocompatibility Complex (MHC, Apanius et al. 1997; Kamiya et al. 2014). This gene-dense genomic region contains genes that encode cell surface molecules, which present both endogenously (Class I type MHC) and exogenously (Class II type MHC) derived antigens to T cells (Janeway et al. 2005). The main function of the MHC is to bind and initiate the elimination of pathogens as part of the adaptive immune system. The MHC is characterised by an exceptionally high diversity of different MHC alleles at the population level and an excess of heterozygosity at the individual level (Stet et al. 2003). Specific MHC alleles can provide parasite-specific resistance and allele combinations can determine overall parasite burden (Penn et al. 2002; Wegner et al. 2003*a*; Eizaguirre et al. 2009*b*, 2012*b*). MHC-based female mate choice for specific alleles follows the good gene theory (e.g. Lohm et al. 2002; Eizaguirre et al. 2009), whereas mate choice that optimises offspring MHC diversity is based on compatibility (maximal heterozygosity: e.g. (Potts et al. 1991; Consuegra and de Leaniz 2008); optimality: e.g. (Aeschlimann et al. 2003; Bonneaud et al. 2006*a*)). Both are actually not mutually exclusive (Roberts and Gosling 2003; Eizaguirre et al. 2009*b*), since it is speculated that

compatible genes can be assessed via odour signals from afar, whereas honest signals indicate specific good genes upon closer inspection (Mays and Hill 2004; Jäger et al. 2007).

Female mate choice in three-spined stickleback (*G. aculeatus*) is MHC-based for both good and compatible genes (Milinski et al. 2005; Eizaguirre et al. 2009*b*). Male sticklebacks indicate body condition and parasite infection through the intensity of their red throat colouration (Milinski and Bakker 1990; Bakker and Mundwiler 1992). Mate choice for bright males is therefore a choice for individuals with good genes expressed as resistance towards currently prevalent parasite species (Jäger et al. 2007; Eizaguirre et al. 2009*b*). Overall, parasite resistance and, consequently, fitness, is highest at intermediate individual MHC diversity (Wegner et al. 2003*a*, 2008; Kalbe et al. 2009). Accordingly, female use self-reference and count their mates' MHC alleles to optimise MHC diversity for their offspring (Reusch et al. 2001*a*; Aeschlimann et al. 2003; Milinski et al. 2005). Whereas the optimal individual MHC allele diversity only varies slightly between years within a population but can vary among populations, the resistance advantage of single MHC variants differs considerably over time and space (Eizaguirre et al. 2009*b*; Lenz et al. 2009*b*; Eizaguirre et al. 2012*b*; Andreou et al. 2017).

We designed a two-phase field experiment in order to i) identify specific MHC alleles conferring resistance against predominant parasites, ii) test whether the presence, and respectively the absence, of resistance allele results in different mate choice behaviour and alters the strength of sexual selection as consequence. Specifically, using field mesocosms (Eizaguirre et al. 2012*a*), we exposed laboratory-bred three-spined sticklebacks with distinct MHC alleles to their natural parasite community and identified those alleles conferring resistance against specific parasites. We then selected fish families which showed segregation of for these MHC alleles to create replicated treatment groups where fish shared a common genetic background but differed by the presence or absence of the resistance MHC alleles. These treatment groups were released into enclosures located in the lake of origin of the fish where they were exposed to a natural parasite diversity (Eizaguirre et

al. 2009*b*). We assessed mate choice and individual lifetime reproductive success using parenthood analysis (Kalbe et al 2009) as well as parasite load and other fitness proxies. We tested the hypothesis that female choice for compatibility will be found when additive genetic benefits from good genes are absent, resulting in little variation in reproduction between the sexes as consequence. When good genes are present female choice will be based on these good genes given that they provide significant fitness benefits to the parental generation. This should lead to increased variation of reproductive success between sexes in the treatment groups with good genes, an element typically associated with strong sexual selection.

**Material and Methods**

*PHASE 1 – FISH BREEDING AND CAGE SET-UP*. We bred 13 three-spined stickleback (*G. aculeatus*) families from 26 random wild-caught fish collected from the Großer Plöner See (54°9'21.16'N, 10°25'50.14'E, Germany) in August 2016. After 10 months, a total of 20 fish per family were transplanted to 10 stainless steel cages (Length: 100cm; width: 60cm; Height 25cm) and placed in their native lake, experiencing parasite exposure for the first time (Eizaguirre et al. 2012*a*). The cages were based 15 meters offshore at 1-1.5m depth, separated by ~ 50cm. The fish were recovered in the 6$^{th}$ week, two cages at a time per day. All fish were measured, weighed and screened for ecto- and endo-parasites (Kalbe et al. 2002). We used fingerprinting from spine and fin clips to re-identify individuals at the end of the experiment (Kalbe et al. 2009).

*MICROSATELLITE AND MHC TYPING*. We extracted DNA from spines and fins using DNeasy 96 Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. All fish were genotyped for nine microsatellites combined in two different multiplexed PCRs (Kalbe et al. 2009). MHC class II*β* genotyping was determined using reference-strand-mediated conformation analysis (RSCA, Lenz *et al.* 2009a). We targeted the exon II of the MHC region which encodes the highly variable peptide-

binding beta chain region of the final MHC molecule. The MHC II*β* genes in stickleback are duplicated and frequently organised in tightly linked alleles, which we refer to as haplotypes (Lenz et al. 2009*a*). From this we assigned individual MHC genotype as well as determined individual MHC diversity.

*PHASE 2 - ENCLOSURE EXPERIMENT*. Based on the field experiment results, we selected five out of the 13 original families which segregated differently for a resistance MHC haplotype. This allowed us to test whether sexual selection dynamics differ between treatment groups with fish carrying resistance MHC haplotypes or not. We spine-clipped all individuals of these five families and determined MHC and microsatellite genotypes. We determined fish sex visually and selected two females and males from three families with resistance-associated MHC haplotypes and two of each sex from the same family with the alternative MHC haplotypes. These were then grouped in i) three populations with resistance MHC haplotypes and ii) three populations without the resistance MHC haplotypes. Those six groups were then assigned randomly to one of six stainless steel enclosures (3x3m, height of 1m, 0.4-0.6m above the water surface, 0.5cm mesh size) situated in the original lake of the fish's parents (Eizaguirre et al. 2009*b*; Kalbe et al. 2009). These enclosures are constructed to allow the passage of stickleback prey, parasites and intermediate parasites' hosts. They further allow sticklebacks to engage into natural reproductive behaviours (e.g. territoriality, nest building, female mate inspection), while being protected from avian predation by a net.

*FISH PARASITE LOAD & BODY CONDITION*. We recovered the fish after 45 days in the enclosures. The surviving fish were dissected and screened for all ecto- and endo-macroparasites (Kalbe et al. 2002). In addition, we calculated an individual parasite index ($I_{PI}$), combining parasite diversity and abundance of all parasites (Kalbe et al. 2002). Each fish was measured, weighed and fin-clipped for re-identification. Gonad and testis weight were taken. Initial and final weight and

length were used to calculate initial and final body condition as $BC = \left(\frac{weight}{length}\right)^{b} \times 100$ with *b* fixed at 3.0 (Frischknecht 1993).

*EGG COLLECTION AND INDIVIDUAL LIFETIME REPRODUCTIVE SUCCESS*. Each enclosure was inspected for nests on a weekly basis. We carefully removed all egg clutches, randomly collected twenty-four eggs of each clutch for DNA extraction and parenthood analysis (Eizaguirre et al. 2009*b*; Lenz et al. 2009*b*). DNA extraction was performed on a Freedom evo robot (Tecan) using Invisorb Tissue HTS 96 kit/R (Stratec). A total of 99 clutches were collected and 2 281 eggs were genotyped for parenthood analysis based on nine microsatellites using the software PAPA (Duchesne et al. 2002; Eizaguirre et al. 2009*b*). We assigned parents to 94.4% of all eggs unambiguously and thus, determined individual lifetime reproductive success (LRS) for all fish and nest ownership for all males (Eizaguirre et al. 2009*b*). Alternative male sneaking behaviour was identified when a male other than the nest owner fertilised some eggs within a clutch (Kalbe et al. 2009). Only eggs sired by nest owners are a result of female choice. Eggs were counted as stolen when they were neither sired by the nest owner nor laid by a female that mated with the nest owner.

*DATA ANALYSES*. All statistical analyses and graphical visualizations were done with R version 3.3.1 (R Core Team 2016,packages include: '*vegan*', '*ggplot2*', '*lmerTest*', '*lme4*'). Model residuals were tested for normality and homoscedasticity and data was transformed if required to meet tests' assumptions. All models were backward-selected using the *anova* function.

*PHASE 1 - LINKING MHC AND MACROPARASITE DIVERSITY*. To identify which MHC haplotypes was associated to resistance against specific parasite species, we split the three most common parasite species (Glochidia, *Diplostomum* sp, *Camallanus lacustris*) into three infection groups (high, intermediate, low). We performed a PERMANOVAs with MHC haplotype matrix as response and high versus low infection group as explanatory variable and cage as a block factor. Since the MHC haplotype matrix is a presence/absence matrix we used Jaccard distance. We ran a similarity

percentage analysis (SIMPER), where a significant PERMANOVA result was found, to identify the specific MHC haplotypes. Lastly, we used a mixed effect model to confirm the effect of the identified MHC haplotypes on fish parasite load for the specific parasites. We added initial body condition (calculated as (weight/length)$^b$x100 with *b* being set at 3.0; Frischknecht 1993) and MHC haplotype zygosity as co-variable and assessed their interaction. Family and cage were set as random factors. We used false discovery rate correction in case of multiple testing ($p_{fdr}$= p-value after correction).

*PHASE 2 – MATE CHOICE, LIFETIME REPRODUCTIVE SUCCESS AND THE STRENGTH OF SEXUAL SELECTION*. In order to determine the degree of self-reference females used during mate choice, we calculated an MHC variant-sharing value between females and males from the same enclosure (Wetton et al. 1987; Eizaguirre et al. 2009*b*):

$$D = \frac{2F_{ab}}{F_a + F_b}$$

Where $F_{ab}$ is the number for MHC variants shared and $F_a$ and $F_b$ is the sum of MHC variants for individual *a* and *b*, respectively. We then simulated 1000 random mate choice events with respect to the MHC variant sharing value among all reproductively active males. We resampled 1000 times from the observed mate choice and compared the simulated to the observed MHC variant sharing value for each enclosure with a Wilcoxon rank-sum test (Eizaguirre et al. 2009*b*). Only if mate choice was not random with regards to the MHC variant sharing value, we also determined the direction of MHC-based mate choice, i.e. assortative or dis-assortative. We compared the observed MHC haplotype sharing value first with a simulated choice for the most MHC-dissimilar individuals using a Wilcoxon rank-sum test (Eizaguirre et al. 2009*b*). We also investigated whether in the populations with an MHC haplotype linked to parasite resistance, female choice was directed towards that MHC haplotype the same resampling approach described above comparing observed to random choice over 1000 permutations.

We the used a linear mixed effect model to test for the effect of initial body condition, fish sex, individual parasite load ($I_{PI}$), treatment group, the resistance associated MHC haplotype(s) and their interactions on individual lifetime reproductive success. Enclosure and fish family were set as random effects. To test for variance in reproductive success, we used a Levene's test between sexes – an indicator for the strength of sexual selection – and between males from different treatment groups.

*PHASE 2 – ALTERNATIVE MATING TACTICS*. After determining the number of eggs fertilised by sneaking, we compared the proportion of sneaked egg between the treatment groups using a student's t-test. We estimated the effect of MHC haplotype, initial body condition, $I_{PI}$, treatment and their interactions on the proportion of eggs fertilised by male sneaking behaviour (log-transformed) using a generalised linear mixed effect model with family and enclosure as random effects. Lastly, we calculated a gonadosomatic index for males (testis mass/fish weight) and used the log-transformed index in a linear mixed effect model with MHC haplotype, final body condition, $I_{PI}$, treatment group and their interactions as explanatory variables and family and enclosure as random effects.

*PHASE 2 – PARASITE RESISTANCE AND FINAL BODY CONDITION*. To explain the effects of MHC haplotypes on mate choice and LRS, we first ran a linear mixed effect model to test the effect of treatment group, initial body condition, sex and the presence of the resistance associated MHC haplotype on the log-transformed number of *Diplostomum* sp. found in the fish eyes as this parasite revealed to correlate with MHC. Here as well enclosure and family were set as random effects. Second, we used a similar mixed effect model to estimate the effect of sex, initial body condition, treatment group and MHC haplotype(s) and their interactions on log-transformed $I_{PI}$. To test whether the resistance to specific parasites is associated to differences in the parasite community between treatment groups, we compared parasite communities using a PERMANOVA with enclosures set as a block factor. In order to test whether parasite-specific MHC-based resistance also benefited the host by reducing coinfection with other co-occurring parasites we used a PERMANOVA comparing

parasite communities between groups of fish infected with few or many of the specific parasite species. Enclosure was treated as block factor.

Lastly, we ran a linear mixed effect model on final body condition using sex, $I_{PI}$, treatment group and MHC haplotype(s) and all their interactions as explanatory variables as well as enclosure and family set as random effects.

**Results**

*PHASE 1 - LINKING MHC AND MACROPARASITE DIVERSITY*. After 6 weeks in the cages, we dissected fish and found that haplotype No05 (allele No05, NCBI accession number AY687829) was associated with fewer *Diplostomum* sp. ($F_{1,243}$=18.20, $p_{fdr}$=0.0004; Supplementary Table 1a, Supplementary Table 2), a trematode parasite that infects stickleback and reaches the eye lens within a short period post exposure (Rauch et al. 2006). By contrast, MHC haplotype No15.No16 (alleles No15 and No16, NCBI accession number DQ016410 and DQ015617, respectively) was associated with increased susceptibility to *Diplostomum* sp. ($F_{1,92}$=7.58, $p_{fdr}$=0.014, Figure 1A, Supplementary Table 1a, 2). We also identified individuals with MHC haplotype No36No54 (alleles No36 see NCBI accession number DQ016411, No54, Appendix 1) to be more resistant towards the nematode *Camallanus lacustris* ($F_{1,60}$=7.11, $p_{fdr}$=0.030, Figure 1B; Supplementary Table 1b, 2). Infection with the generalist fish parasite Glochidia was not associated with any MHC haplotypes (PERMANOVA: $F_{1,160}$=1.45, p=0.145).

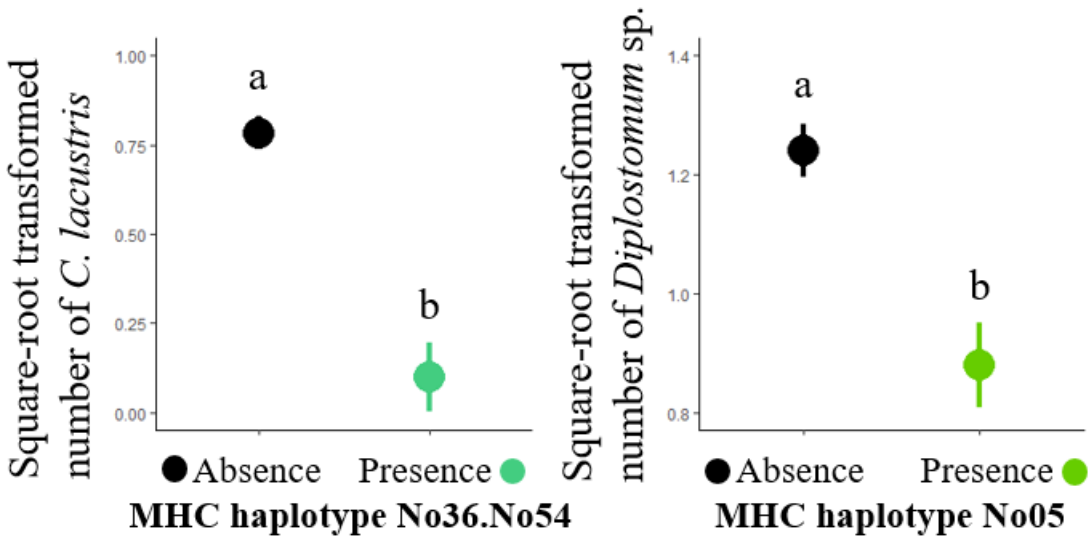



**Figure 1 A) Individuals with haplotype No36.No54 (turquoise) had fewer *C. lacustris* (square-root transformed; mean ± standard error). B) Individuals with haplotype No05 (green) harboured fewer *Diplostomum* sp.** Letter depict significant main effect.

*PHASE 2 – MATE CHOICE, LIFETIME REPRODUCTIVE SUCCESS AND THE STRENGTH OF SEXUAL SELECTION*. Upon the discovery of two resistance-associated MHC haplotypes, we predicted that in populations with individuals carrying these haplotypes females would choose males with these haplotypes. On the other hand, in populations without these haplotypes mate choice for compatible genes should be observed.

On average, females produced a comparable number of clutches between treatment groups (random pop.: mean 13.3 ±2.0SE=standard error; resistant pop.: 20.0 ±3.3SE; t-test: p=0.222). In the treatment groups without males carrying good genes (i.e. the resistance associated MHC haplotype), female choice was not random (Wilcoxon rank-sum test, 3 out of 3 p-values<0.05; Supplementary Table 3a). Female choice was directed towards an optimal intermediate MHC number for the offspring since it was different from choice for the most dissimilar (3 out of 3 p-values<0.05, Supplementary Table 3a) and similar individuals (3 out of 3 p-values<0.05, Supplementary Table 3a). Amongst all enclosures with individuals with the resistance-associated haplotype No05, females chose irrespective of MHC

compatibility (3 out of 3 p-values>0.1, Supplementary Table 3b), instead females chose males carrying the haplotype No05 (3 out of 3 p-values<0.001, Supplementary Table 3b). MHC haplotype No36.No54 was not associated with MHC-based mate choice for this specific haplotype (2 out of 3 p-values>0.1, Supplementary Table 3b).

As expected, individual lifetime reproductive success was highest for individuals with haplotype No05 ($F_{1,21}$=5.49, p=0.029) and was positively related to initial body condition ($F_{1,95}$=6.89, p=0.010), but did not differ between sexes and treatment groups (Table 1). Furthermore, the variance in reproductive success (number of eggs) between males and females differ in populations where fish carried the resistance associated MHC haplotype (Levene's test, n=60, p=0.013). By contrast, variance in life time reproductive success was not different between males and females in populations without haplotype No05 (Levene's test, n=60, p=0.216). The variance in lifetime reproductive success was similar between males from different treatment groups (Levene's test, n=60, p=0.405).

**Table 1. Summary table of a linear mixed effect model on lifetime reproductive success using treatment group, MHC haplotype No05, initial body condition and sex as explanatory variable and fish family and enclosure as random effects.** Significant results are highlighted in bold. *d.f.* denotes degrees of freedom. *accounted for presence/absence in replicate populations.

| **Lifetime Reproductive Success** | *d.f.* | F-value | p-value |
|---|---|---|---|
| **Treatment group** | 1,6 | 1.25 | 0.307 |
| **Initial body condition corrected for sex** | 1,94 | 6.31 | **0.014** |
| **Sex** | 1,85 | 2.19 | 0.143 |
| **MHC haplotype No05*** | 1,21 | 4.80 | **0.040** |
| **Individual Parasite Load** | 1,101 | 0.42 | 0.517 |

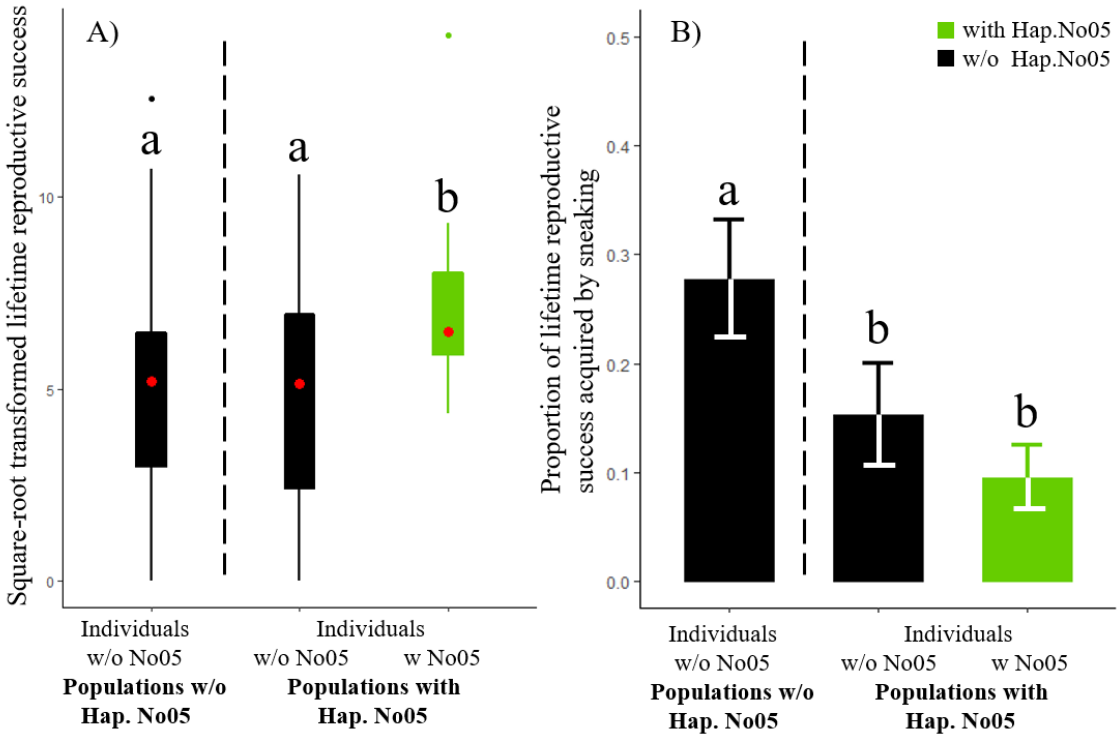



**Figure 3 A) Individual lifetime reproductive success (square-root-transformed) between treatment groups and individuals with (green) and without (black) Haplotype No05. B) Mean proportion of eggs fertilised via sneaking behaviour (±SE) comparing treatment groups and individuals with and without haplotype No05.** Mean shown as red point with box showing upper and lower quartiles. Letter depict significant main effect.

*PHASE 2 – ALTERNATIVE MATING TACTICS*. In the treatment groups without fish carrying the resistance associated MHC haplotype, proportionally more eggs were fertilised via sneaking (random pop.: mean 27.0% ±5.8 standard error=SE; resistant pop.: 12.8% ±3.1SE; t-test: p=0.039), while the number of eggs stolen was similar (random pop.: mean 1.3 ±0.5SE; resistant pop.: 2.5% ±0.8SE; t-test: p=0.195). The individual proportion of eggs sired by sneaking was also negatively associated with initial body condition ($x^2_1$=27.59, p<0.001, Supplementary Table 4). An interaction between parasite load and treatment group ($x^2_1$=9.63, p=0.002) revealed that males from those populations with haplotype No05 sneaked less but increasingly more at higher parasite load, while in the absence of resistance associated MHC haplotype, males sneaked at equal frequency regardless of parasite

load. Lastly, the gonadosomatic index was higher for those males with haplotype No05 ($F_{1,46}$=4.13, p=0.048, Supplementary Table 5). But what caused differences in sneaking behaviour and mate choice between treatment groups with and without individuals having MHC haplotype No05?

*PHASE 2 – PARASITE RESISTANCE AND FINAL BODY CONDITION*. Individuals with MHC haplotype No05 had reduced infection load of *Diplostomum* sp. compared to individuals lacking this haplotype (Figure 3A; Table 2a; $F_{1,25}$=5.37, p=0.029). The abundance of this eye fluke was positively associated with initial body condition of the fish ($F_{1,97}$=6.83, p=0.010) and was higher in males ($F_{1,81}$=6.38, p=0.013). *Diplostomum* sp. load did not differ between treatment groups (Table 2a). Overall parasite load ($I_{PI}$) was not different between treatment groups, nor amongst individuals with and without the haplotype No05 (Supplementary Table 6), as predicted by the specific function of MHC molecules. The parasite community did not differ between treatment groups ($F_{1,105}$=1.09, p=1). In terms of coinfection, *Diplostomum* sp. were significantly more likely to co-occur with other parasite species ($F_{1,69}$=5.98, p=0.001): High *Diplostomum* sp. infection was associated with high number of *Cyathocotyle prussica* (Similarity Percentage Analysis; p=0.001; Supplementary Table 7) and the eye fluke *Apatemon* sp. (p=0.003) as well as marginally more frequent with *Tylodelphis clavata* (p=0.078). Since *Diplostomum* sp., *Apatemon* sp. and *T. clavata* share infection pathways and the same parasite life cycle, we developed an eye fluke index calculated following the same method as for the $I_{PI}$ (Kalbe et al. 2002). We estimated the effect of treatment group, sex, initial body condition, MHC haplotype No05 and their interactions on the eye fluke index (log-transformed) with enclosure and family as random effects. We found that males ($F_{1,79}$=4.90, p=0.030) and individuals with No05 ($F_{1,23}$=4.89, p=0.037) had a lower index (Table 2b). Initial body condition was positively associated with the eye fluke index ($F_{1,95}$=8.74, p=0.004)

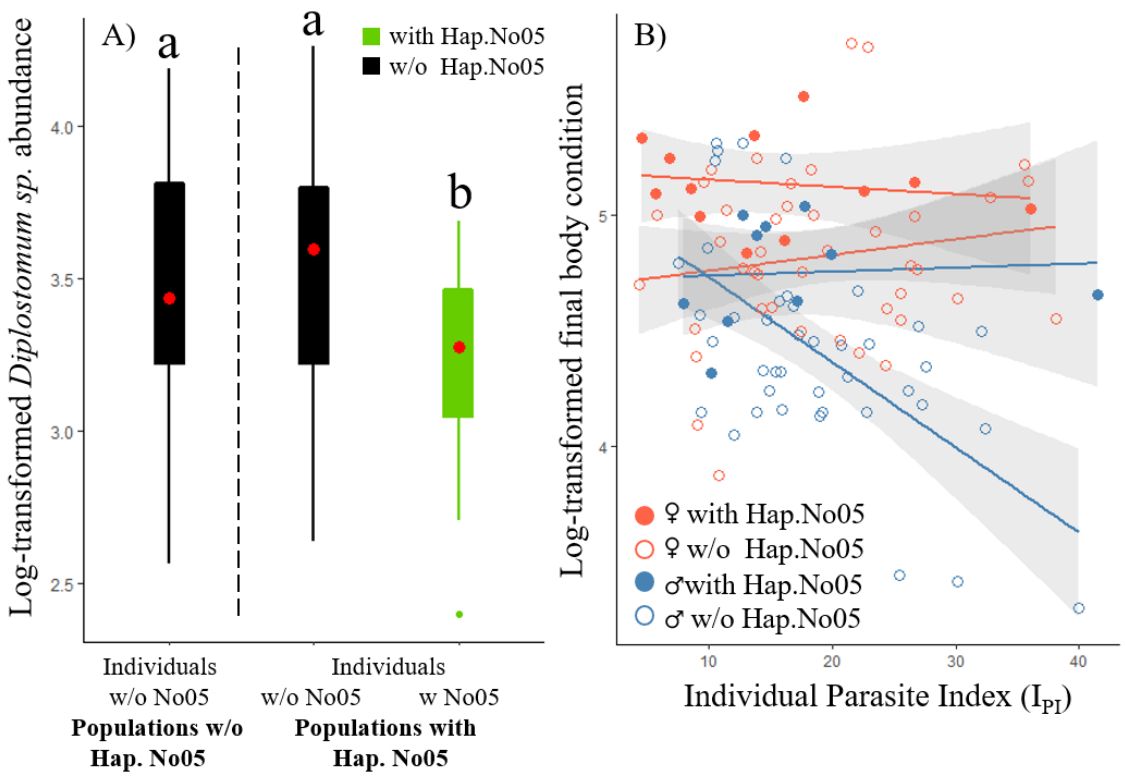



**Figure 3 A) *Diplostomum* sp. abundance (log-transformed) between treatment groups and individuals with (green) and without (black) Haplotype No05. B) Final body condition in relation to parasite load ($I_{PI}$), sex (red=female, blue=male) and presence of Haplotype No05 (empty circles=absent; filled circles=present).** Mean shown as red point with box showing upper and lower quartile. Letter depict significant main effect. Three-way interaction between sex, presence of the haplotype No05 and parasite load was still visible when outlier ($I_{PI}$>40) was removed ($F_{1,104}$=3.60, p=0.060).

We found a three-way interaction between sex, individual parasite load and the presence of MHC haplotype No05 ($F_{1,98}$=7.30, p=0.008; Table 2c): The body condition of female fish with and without haplotype No05 did not change with increased parasite load. Similarly, male fish with haplotype No05 tolerated increased infection without a reduction in body condition, but males without No05 suffered sharply reduced body condition at higher parasite load (Figure 3B). Given the strong effect of sex, we re-ran each model separately for each sex: Haplotype No05 was associated with increased female body condition ($F_{1,24}$=11.98, p=0.002; Supplementary Table 8) and an interaction between

haplotype No05 and parasite load ($F_{1,43}$=8.26, p=0.006) reiterating that males with No05 tolerated overall parasite infection better than those lacking it (Figure 3B).

**Table 2. Statistical Summaries of linear mixed effect models on a) log-transformed *Diplostomum* sp. load, b) the eye fluke index using treatment group, MHC haplotype No05, initial body condition and sex as explanatory variable and another model c) on final body condition with treatment group, MHC haplotype No05, $I_{PI}$ and sex as explanatory variables. Fish family and enclosure were set as random effects.** Models were backward selected using the *anova* function. Significant results are highlighted in bold. d.f. denotes degrees of freedom. *accounted for presence/absence in replicate populations.

| **a) *Diplostomum* sp. load** | ***d.f.*** | **F-value** | **p-value** |
|---|---|---|---|
| **Treatment group** | 1,8 | 0.07 | 0.796 |
| **MHC haplotype No05*** | 1,25 | 5.37 | **0.029** |
| **Initial body condition corrected for sex** | 1,97 | 6.83 | **0.010** |
| **Sex** | 1,81 | 6.38 | **0.013** |
| | | | |
| **b) Eye Fluke Index** | | | |
| **Treatment group** | 1,5 | 0.01 | 0.932 |
| **MHC haplotype No05*** | 1,23 | 4.89 | **0.037** |
| **Initial body condition corrected for sex** | 1,95 | 8.74 | **0.004** |
| **Sex** | 1,79 | 4.90 | **0.030** |
| | | | |
| **c) Final Body Condition** | | | |
| **Treatment group** | 1,30 | 0.44 | 0.510 |
| **MHC haplotype No05*** | 1,91 | 0.16 | 0.690 |
| **Individual Parasite Load** | 1,104 | 11.40 | **0.001** |
| **Sex** | 1,97 | 1.65 | 0.202 |
| **MHC haplotype No05 * Individual Parasite Load** | 1,105 | 2.16 | 0.145 |
| **MHC haplotype No05 * sex** | 1,95 | 6.69 | **0.011** |
| **Individual Parasite Load * sex** | 1,96 | 17.57 | **<0.001** |
| **MHC haplotype No05 * sex * Individual Parasite Load** | 1,98 | 7.30 | **0.008** |

**Discussion**

MHC-based mate choice is common amongst animals, including fish (Milinski et al. 2005), birds (Bonneaud et al. 2006*a*) and humans (Wedekind et al. 1995), but how changes in the immunogenetic make-up of a population affects sexual selection remains an open question. By *a priori* identifying MHC haplotype associated with increased parasite resistance, we hypothesized that two different sexual selection dynamics should be visible in populations exposed to similar parasite communities. We predicted that in the presence of resistance MHC haplotype in a population, females should favour MHC-based mate choice for good genes, and therefore we expected strong sexual selection via mate choice in the form of differential male reproductive fitness. On the other hand, in the absence of resistance MHC haplotypes, we predicted female mate choice for MHC compatibility, resulting in lower variance in male reproductive success and therefore reduced strength of sexual selection.

Exposing fish with a diverse set of MHC haplotypes to their native parasite communities, we identified specific MHC haplotypes associated with resistance against *Diplostomum* sp. and *C. lacustris*. Specifically, MHC haplotype No05 was associated with a significant reduction in *Diplostomum* sp. infection. Unless the fish host resists infection, the actively infecting trematode *Diplostomum* sp. invades the immunologically protected eye lens within 24 hours (Rauch et al. 2006). A large number of *Diplostomum* sp. can visually impair the host, lowering its foraging efficiency and anti-predator behaviour (Crowden and Broom 1980). Additionally, this generalist trematode often co-occurs with other parasite species likely due to shared transmission pathways and life history (Benesh and Kalbe 2016).

Following our predictions, in the absence of fish carrying the resistance associated MHC haplotype No05, females chose males with an MHC genotype that, combined with her own, resulted in an optimally intermediate number of MHC alleles for their offspring. This finding is consistent with the compatibility hypothesis (Reusch et al. 2001*a*; Milinski et al. 2005; Andreou et al. 2017). In comparison, we find that when males with MHC haplotype No05 were present, females preferentially

mated with them. This resulted in increased reproductive success of those individuals with No05 – a typical pattern of mate choice for good genes (Kokko 2001). Mate choice also lead to skewed male reproductive success compared with that of females, indicating intense sexual selection when good genes are present (Andersson 1994). This is because in a population with large differences in functional advantages amongst alleles, females should preferentially choose males with good genes, i.e. alleles of high intrinsic quality, leading to strong directional sexual selection for the good gene (Colegrave et al. 2002; Neff and Pitcher 2005). Vice versa, when non-additive genetic benefits of allelic diversity are high, a strategy to choose compatible mates may be advantageous (Neff and Pitcher 2005) because optimal MHC allele diversity can increase overall parasite resistance (Wegner et al. 2003*a*). We corroborate these theoretical predictions. In addition, previous enclosure experiments found females choice for specific MHC haplotypes only under strong resistance benefits (Eizaguirre et al. 2009*b*; Lenz et al. 2009*b*). Together, these findings suggest mate choice is context-dependent with regards to the genetic make-up of a population.

Interestingly, males fertilised fewer eggs via sneaking behaviour when fish with good genes were present and fish with haplotype No05 had higher relative gonad weight. This suggests that males with No05 have better competitive capacity. As such our findings support the hypothesis that while fending off competitors to ensure their own reproductive output (Largiader et al. 2001) and investing in sperm quality (Kaufmann et al. 2015), high quality males engage less in alternative, i.e. risky fertilisation tactics (Engqvist et al. 2014). In fact, this portrays alternative fertilisation tactics to be similarly context-dependent as mate choice (Engqvist and Taborsky 2016). Like in the case of mate choice, density-mediated effects and antagonistic selection can impact male fertilisation tactics: Increased male density, for instance, leads to more sneaking behaviour (Eizaguirre et al. 2009*b*) and reduced visibility and, therefore lower detection risk, to more sneaking attempts (Candolin and Vlieger 2013). From our experiment, we suggest that not only female choice, but male fertilisation tactics are context-dependent with regards to the presence of good genes. This however, did not lead to

signatures of stronger sexual selection, likely because fertilisation by sneaking only supplements individual lifetime reproductive success acquired by female choice. But what are the exact fitness benefits associated with haplotype No05?

Individuals with MHC haplotype No05 had reduced *Diplostomum* sp. infection and lower rates of coinfection with other eye flukes with shared transmission pathways and life cycles (Lotz et al. 1995). As a consequence, female fish with haplotype No05 maintained a higher body condition, explaining their high lifetime reproductive success. Males with haplotype No05 sustained a high body condition in spite of increasing parasite load, suggesting increased tolerance to also be associated with MHC haplotypes (Raberg et al. 2007). Since body condition is a major determinant of male-male competition (Dufresne et al. 1986; Largiader et al. 2001) and expression of sexual signals (Milinski and Bakker 1990) males with the resistance associated MHC haplotype competed and defended their territories more successfully and attracted more females. Combined, this resulted in choice for and increased lifetime reproductive success of individuals with this haplotype.

By contrast, mate choice was likely not directed towards MHC haplotype No36.No54, which was associated with resistance against *Camallanus lacustris* in the field experiment, because the MHC haplotype did no longer provide a substantial fitness advantage at the time mating occurred. This could be either due to changes in the abundance of *C. lacustris* or changes in the relationship between MHC haplotype and infection intensity (Chapter 5). This underscores that the relationship between MHC haplotype and parasite resistance undergoes temporal variation in strength, but crucially at reproduction that link is most evolutionarily relevant.

What are the broader implications of context-dependence female mate choice? What differentiates mate choice for compatible genes from that for good genes, is that compatible choices result in an optimal individual MHC diversity for the offspring, whereas choice for good genes provides the individuals and their offspring increased fitness if the environmental conditions remain stable

(Milinski 2015). This is because optimal intermediate diversity leads to reduced parasite load (Wegner et al. 2003*a*), higher survival (Wegner et al. 2008) and reproductive output (Kalbe et al. 2009) in the next stickleback generation. By contrast, choice based on good genes, i.e. specific alleles, only benefits offspring if the selection pressure is constant or predictable (Eizaguirre et al. 2012*b*). As a consequence, specific MHC haplotypes under positive natural selection and mate choice propel the rapid frequency turn-overs between generations (Eizaguirre et al. 2009, 2012*a*; Lenz et al. 2009*b*). In that, context-dependent female choice for good genes will accelerate parasite-mediated negative-frequency dependent selection on MHC haplotypes. Thus, we suggest that the presence of high quality good genes can operate a switch between mate choice for good genes, compatible genes or both.

**Acknowledgment**. We like to thank I. Schultz, G. Schmiedeskamp and R. Derner for their technical support throughout the project and D. Martens for helping with fish rearing.

# CHAPTER 7. HOST-PARASITE COEVOLUTION IN SPITE OF CONCOMITANT SELECTION

*Summary.* The main objective of this thesis was to characterize how different levels of parasite-mediated natural and sexual selection interact with other concurrent selection pressures and modulate host-parasite coevolution. I (and my colleagues) tackled this by looking specifically into temporal and spatial variation in parasite-mediated selection, as well as the impact of predation and variation in the host's genetic make-up on the evolution of parasite resistance via natural and sexual selection (Figure 1). Collectively, the results depict just how context-dependent host-parasite interactions are in nature.

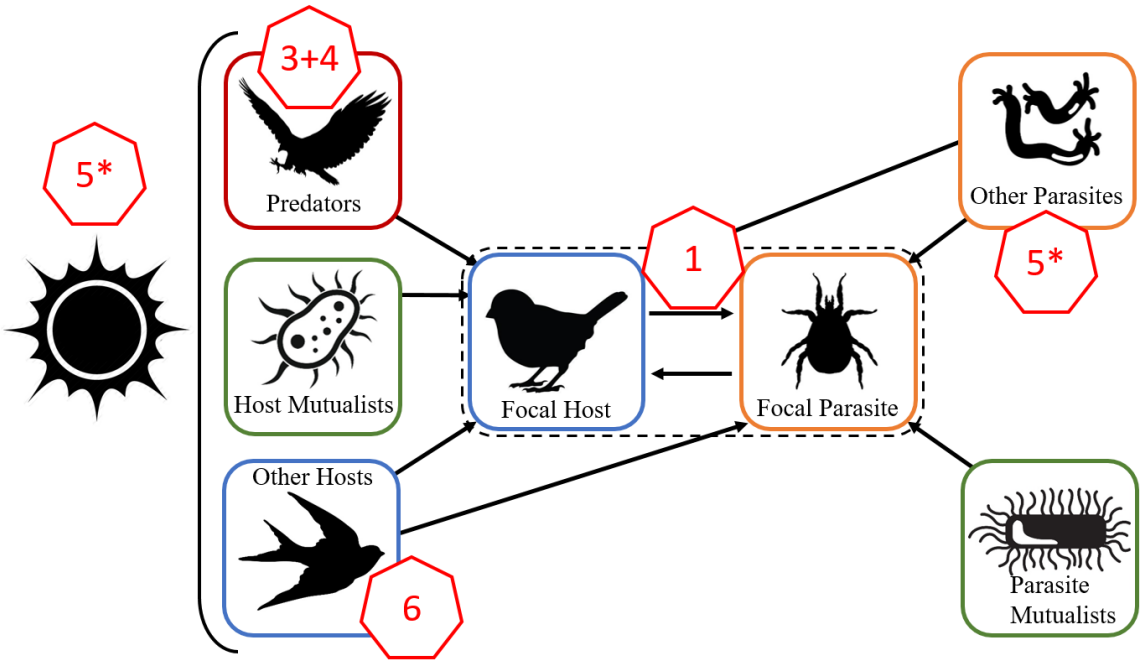



**Figure 1. Host-parasite coevolution occurs in a complex environment with multiple concurrent selection pressures. The thesis addressed several of selection pressures.** Red heptagons with numbers show which concurrent selection pressure we examine and in which chapter. * indicated chapter that covers multiple aspects simultaneously.

Host-parasite interactions are considered a major driver of evolutionary change and biological diversification (Thompson 1994; Poulin 2007). Adaptations to avoid, tolerate and resist parasite infection vary widely amongst hosts (Klein and Figueroa 1986; Behringer et al. 2006; Raberg et al. 2007). Recurrent selective sweeps of novel adaptations or the cycling in frequency of a diverse set of resistance alleles in accordance with currently prevalent parasites are two common mechanisms for the evolution of resistance (Chapter 1). In addition, parasite-mediated sexual selection and especially mate choice can generate gene combinations of increased fitness or select for variants of particular quality (Hamilton and Zuk 1982; Tregenza and Wedell 2000). Yet, little is understood about how concomitant selection pressures, such as predation (Betts et al. 2016), or variation in the hosts genetic make-up affects the dynamics underlying the evolution of host resistance via natural and sexual selection driven by parasites (Figure 1, Chapter 1).

In vertebrates, recurrent exposure to parasites has led to the evolution of the adaptive immunity, which primarily relies on immunological memory to identify and eliminate parasites upon re-exposure. However, establishing immunological memory must come at a cost (Sheldon and Verhulst 1996). Trade-offs can be in the form of reduced survival, condition, growth (Lochmiller and Deerenberg 2000; Tschirren and Richner 2006) or reproductive fitness (Bonneaud et al. 2003). Using the three-spined stickleback as the model organism, I was able to show that acquired immunity is costly to those fish that did not experience parasite re-infection, whereas immunological memory benefited those repeatedly exposed to the same parasite species (Figure 1, Chapter 2). These findings are an extension to previous work because the results are based on individual lifetime reproductive success rather than proxies of reproductive success as fitness measure and show both, benefits and costs simultaneously (Chapter 2). Parameterising an illustrative population-based adaptive model with results from our experiment revealed that a 10% benefit of immunity under recurrent parasite infection results in the rapid fixation of this trait (i.e. adaptive immunity, Chapter 2). Such results explain the diversity of locally optimised immunity by host populations as a response to trade-offs between immunity, infection and Darwinian fitness (Eizaguirre et al. 2012*a*; Lenz et al. 2013; Huang et al. 2016).

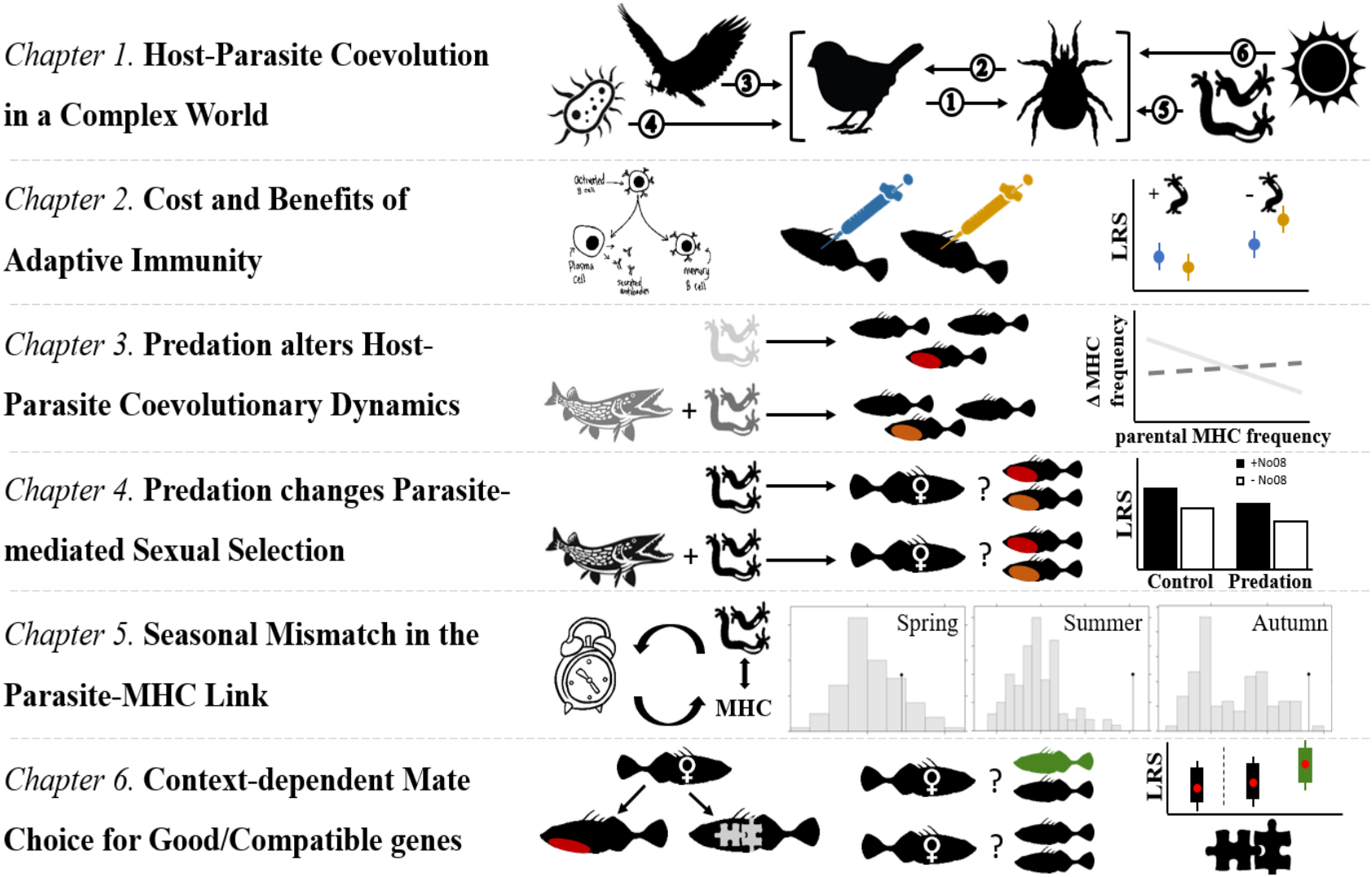



**Figure 1. Graphical summary of introductory chapter and all data chapters.**

Since the best known genetic basis of parasite resistance and mate choice are the genes of the Major Histocompatibility Complex (Chapter 1), I then questioned whether concomitant selection and temporally variable selection impacts coevolutionary dynamics between host and parasite. Specifically, I tested how predation impacts parasite-mediated natural (Chapter 3) and sexual selection (Chapter 4). Predation pressure is theoretically predicted to strengthen parasite-mediated selection if predation was to remove the most infected, i.e. weakest individuals from the population (Lafferty and Morris 1996; Best 2018). Opposite to our initial expectation, under parasite-mediated selection, but in the absence of a predator, negative frequency-dependent selection on MHC haplotypes was observed (Figure 1, Chapter 3). I showed this by estimating frequency changes of MHC haplotypes across generations using parenthood analysis on stickleback eggs and inferring computationally the frequencies of the codominant MHC haplotypes in the offspring generation (Chapter 3). Since predation also reduced host population density, we hypothesise that predation causes density-dependent eco-evolutionary dynamics between host and parasite, favouring common over rare MHC haplotypes (Huang et al. 2017) and potentially slowing down resistance evolution in the host (e.g. Frickel et al. 2017).

Investigating the implication of predation for parasite-mediated sexual selection on the same data set, we hypothesised predation to affect mate choice behaviour. Female choice in stickleback is based on indirect genetic benefits. These benefits emerge either from the suitable combination of female and male genome, i.e. choice for compatibility, or the intrinsic quality of a mate expressed in condition dependent male signals, i.e. good genes. Comparing random to observed mate choices, I uncover that MHC-based selection for MHC compatibility was not universally present amongst populations when simultaneously exposed to predation (Figure 1, Chapter 4). At the same time, MHC-based mate choice for good genes was observed across all enclosures targeting a MHC haplotype of particularly high resistance benefit (Chapter 4). Together, these two studies illustrated that coevolution under concomitant selection, which is a natural setting, leads to different outcomes, even unravelling parasite-mediated dynamics observed under controlled conditions. In particular, the studies emphasised the role of density-mediated effects on parasite-mediated frequency dependent dynamics. Studies like these surface now more regularly as we

begin to understand that nature is the sum of complex interactions (e.g. Brunner et al. 2017; Best et al. 2017).

An often neglected aspect of parasite-mediated selection is its temporal variability. While an extensive knowledge about seasonal and annual variation in parasite prevalence and diversity exists (Altizer et al. 2006), very little is understood about how that impacts the relationship between host immune-genetics and parasites at distinct time points and, therefore, host fitness (Fraser et al. 2010). I transplanted laboratory-reared sticklebacks from a diverse genetic and MHC background into a lake at three distinct time points across a year (Figure 1, Chapter 5). The parasite community and load differed amongst seasons with implications for the link with MHC-based fitness in the host (Chapter 5). Various MHC haplotypes showed variable associations to specific parasite species across the year, while others maintained their resistance benefit and improved host condition and growth (Chapter 5). This study posits that temporal variation in parasites, whether annual or seasonal, can result in similar patterns of divergent natural selection as observed between habitats (Eizaguirre et al. 2012*a*). But unlike divergent selection between habitats temporal variation, such as generated by seasons, may not be persistent and strong enough to favour entirely different MHC pools, but rather creates a mosaic of selection, maintaining MHC polymorphism within populations.

Another source of variation emerges from distinct genetic make-up of host populations. In contrast to density- and parasite-mediated effects (e.g. Boughman 2007; Hayes et al. 2016), the consequences of genetic variation for sexual selection are poorly understood, but are assumed to be context-dependent (Neff and Pitcher 2005). Assembling host populations with and without specific good genes, i.e. those MHC haplotypes associated with resistance benefits to a specific parasite, I found mate choice only for good genes when present (Figure 1, Chapter 6). By contrast, I found choice for MHC compatibility otherwise (Chapter 6). The results confirm that mate choice is context-dependent but predictable with regards to the presence of good genes with high functional benefit (e.g. resistance). Surprisingly, I also showed that genetic variation plays a role in determining alternative fertilisation tactics in males, since I observed more sneaking in populations without good genes (Chapter 6). Importantly, this

work uncovers another variation that plays an underestimated role in determining female mate choice behaviour.

*Future directions.* Research concerning host-parasite coevolutionary has several exciting future directions. One question may be concerned with gaining a better understanding of the interplay between temporal and spatial heterogeneity in determining the evolution of host resistance. As outlined here and in previous work, there is ample evidence that spatial and temporal heterogeneity in the strength of parasite-mediated selection and differences in the parasite community lead to strikingly different investments in immunity (Eizaguirre et al. 2012*a*; Young and MacColl 2017). Using morphological and immunological proxies, expression profiles and immunogenetic diversity, this has been looked at particularly with respect to differences across habitats (Scharsack et al. 2007; Eizaguirre et al. 2011; Lenz et al. 2013; Feulner et al. 2015). We studied differential selection on MHC alleles across seasons, but since we were unable to track evolutionary change from one generation to the next, the importance of temporal variation for the maintenance of MHC polymorphism remains unresolved.Since temporal variation across years and seasons is likely to become more pronounced owing to climate change, this source of variation will gain evolutionary relevance (Bradshaw and Holzapfel 2006). Experimentally this gap can be addressed by modifying length and variation in exposure by different parasite species using replicated host populations with known family and immunogenetic background and tracking changes in the MHC allele diversity over generations.

Yet, another interesting research avenue are eco-evolutionary dynamics of parasite-mediated selection. Contemporary evolution of sexual selection and its consequences have not been explored unequivocally (Svensson and Gosden 2007; Svensson 2019). The MHC forms a particularly interesting genetic basis for understanding those dynamics, since it evolves rapidly (Piertney and Oliver 2006) and links natural and sexual selection in several species including three-spined stickleback. In a recent mesocosm experiment, Brunner and colleagues for instance (2017) show that fish from river and lake environments differ in their resistance to infection by *Gyrodactylus* sp. and as consequence, deplete the prey community differently. This leads to differential survival between the juveniles from the distinct

ecotypes. Introgressing specific MHC haplotypes associated with resistance to this parasite, for instance, between population, mimicking either hybridization or the evolution of novel MHC alleles, may lead to eco-evolutionary feedbacks as consequence selection for condition-dependent male signals that also indirectly indicate resistance.

In fact, during the data collection for chapter 5, I took first exploratory steps towards this idea. Specifically, I explored the differential impact of treatment groups with and without specific MHC alleles associated with resistance (i.e. No05) on their prey community in semi-natural enclosures (see chapter 5 for specifics). In this pilot, I sampled zooplankton before the fish entered the enclosures and then weekly. I also collected samples from outside the enclosures as control measurements. Using a PERMANOVA on zooplankton samples (1000 permutations; bray-curtis distance) with treatment (control, resistant group i.e. group with fish having No05, random group i.e. w/o No05) as fixed factor and week as block factor, we found significant differences between treatments (Figure 2A; $F_{3,123}$=6.71, p=0.002). A follow-up SIMPER (Supplementary Table 1), identified a significantly higher presence of Cyclopoid copepods when fish are absent (Figure 2B; resistant population vs. control; random population vs. control: both p-values<0.05) and a higher number of Daphnia sp. in the random populations compared to those where Haplotype No05 is present (Figure 2C; resistant vs. random population: p-value=0.027).

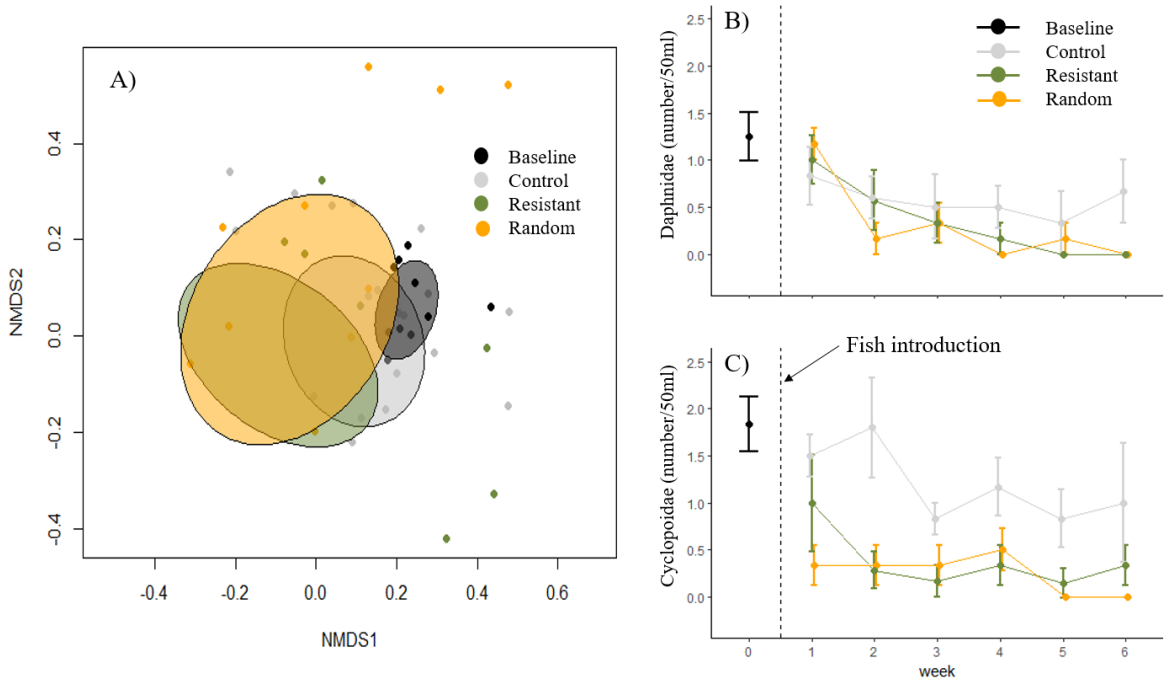



**Figure 2. A) Non-metric multidimensional scaling plot comparing parasite communities across baseline measurements taken before the start of the experiment (black), resistant (with individuals with haplotype No05; green) and random (without No05; yellow) treatment groups and control (grey) samples taken outside the enclosures weekly. B) Weekly measures of Daphnia (mean ±SE=Standard error) and C) Cyclopoid copepods (mean ±SE).**

These findings strengthen previous suggestions that the presence of fish predators impacts prey community (Des Roches et al. 2013; Matthews et al. 2016), but we report such effects for the first time in a semi-natural environment. The lower abundance of cyclopoid copepod prey when fish are present is also in line with field observations and experimental results suggesting that lake fish prefer these nutritious, but highly evasive crustaceans (Lucek et al. 2012; Schmid et al. 2019). The distinct Daphniidae abundances between treatment groups varying in resistance associated haplotypes also suggests that parasite resistance affects the hosts' impact on their prey as already suggested previously using mesocosm experiments (Anaya-Rojas et al. 2016; Brunner et al. 2017). In order to identify feedbacks, such or similar experiments now require an assessment of the consequences emerging from host resistance, which can be viewed as a rapidly evolving trait associated with sexual signal (Svensson 2019). Connecting such

contemporary evolution of a trait under sexual selection with community and ecosystem dynamics across generations will be the challenge for future work.

*Concluding remarks.* The aim of the thesis was to place host-parasite coevolution in a more natural framework. While some chapters followed *a priori* predictions based on theoretical considerations from the literature (Chapter 2, 5, 6), I found unforeseen outcomes (Chapter 3, 4, 6). Crucially, the thesis helps explain why populations differ in their immunocompetence and maintain highly polymorphic, locally adapted MHC allele pools in spite of concurrent selection pressures. With that our work demonstrates the context dependency of host resistance evolution as part of a '*tangled bank*' (Darwin 1859). Besides, the thesis underscores the need for more complex experimental designs to generate an overlap between those predictions arising from laboratory work and those observations gathered from findings in nature. Work on host-parasite coevolution remains a field at the forefront of discovering complex interactions purely based on its intrinsic complexity, but also because of the ubiquity of parasites in nature, the role they play in community and ecosystem dynamics, and the impact they have on human and ecosystem health. Understanding the complex interactions between parasites and host and their environment will help us identify future threats to biodiversity such as through enemy release of invasive hosts (Torchin et al. 2003) or the spread of parasites following human-induced climate change (Pounds et al. 2006). At the same time studying parasite-mediated selection may emphasize what a key feature parasites are for maintaining stable and resilient ecosystems. Particularly with regards to the latter, I genuinely hope that the experimental work presented here will improve our understanding of this intricate and wondrous relationship between hosts and parasites.

# REFERENCES

Acevedo-Whitehouse, K., F. Gulland, D. Greig, and W. Amos. 2003. Disease susceptibility in California sea lions. Nature 422:35.

Acevedo-Whitehouse, K., F. M. D. Gulland, and L. Bowen. 2018. MHC class II DRB diversity predicts antigen recognition and is associated with disease severity in California sea lions naturally infected with *Leptospira interrogans*. Infection, Genetics and Evolution 57:158–165.

Aeschlimann, A. P., M. Häberli, and M. Milinski. 2000. Threat-Sensitive Feeding Strategy of Immature Sticklebacks (*Gasterosteus aculeatus*) in Response to Recent Experimental Infection with the Cestode *Schistocephalus solidus*. Behavioral Ecology and Sociobiology 49:1–7.

Aeschlimann, P. B., M. A. Häberli, T. B. H. Reusch, T. Boehm, and M. Milinski. 2003. Female sticklebacks *Gasterosteus aculeatus* use self-reference to optimize MHC allele number during mate selection. Behavioral Ecology and Sociobiology 54:119–126.

Aguilar, A., and J. C. Garza. 2007. Patterns of historical balancing selection on the salmonid major histocompatibility complex class II beta gene. Journal of Molecular Evolution 65:34–43.

Ahmed, R., and D. Gray. 1995. Immunological Memory and Protective Immunity: Understanding their relation. Science 1285:54–59.

Allesina, S., and S. Tang. 2012. Stability criteria for complex ecosystems. Nature 483:205–208.

Altizer, S., A. Dobson, P. Hosseini, P. Hudson, and M. Pascual. 2006. Seasonality and the dynamics of infectious diseases. Ecology Letters 9:467–484.

Anaya-Rojas, J. M., F. S. Brunner, N. Sommer, O. Seehausen, C. Eizaguirre, and B. Matthews. 2016. The association of feeding behaviour with the resistance and tolerance to parasites in recently diverged sticklebacks. Journal of Evolutionary Biology 29:2157–2167.

Andersen, P. S., and K. Buchmann. 2009. Temperature dependent population growth of

*Gyrodactylus derjavini* on rainbow trout, *Oncorhynchus mykiss*. Journal of Helminthology 72:9.

Andersson, M. 1994. Sexual Selection. Princeton, NJ: Princeton University Press.

Andersson, M., and L. W. Simmons. 2006. Sexual selection and mate choice. Trends in Ecology & Evolution 21:296–302.

Andreou, D., C. Eizaguirre, T. Boehm, and M. Milinski. 2017. Mate choice in sticklebacks reveals that immunogenes can drive ecological speciation. Behavioral Ecology 28:953–961.

Apanius, V., D. Penn, P. R. Slev, R. Ruff, and W. K. Potts. 1997. The Nature of Selection on the Major Histocompatibility Complex. Critical Reviews in Immunology 17:179–224.

Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinants of abundance in parasite communities. Proceedings of the Royal Society B: Biological Sciences 265:1283–1289.

Bakker, T. C. M., and B. Mundwiler. 1992. Female mate choice and male red coloration in a natural three-spined stickleback (*Gasterosteus aculeatus*) population. Behavioural Ecology 5:74–80.

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

Bates, H. W. 1862. XXXII. Contributions to an Insect Fauna of the Amazon Valley. *Lepidoptera: Heliconidæ*. Transactions of the Linnean Society of London 23:495–566.

Behringer, D. C., M. J. Butler, and J. Shields. 2006. Avoidance of disease by social lobsters. Nature 441:421.

Bell, M., and S. Foster. 1994. The evolutionary biology of the Threespine Stickleback. Oxford University Press, New York.

Benesh, D. P., and M. Kalbe. 2016. Experimental parasite community ecology: Intraspecific

variation in a large tapeworm affects community assembly. Journal of Animal Ecology 85:1004–1013.

Bérénos, C., K. M. Wegner, and P. Schmid-Hempel. 2011. Antagonistic coevolution with parasites maintains host genetic diversity: An experimental test. Proceedings of the Royal Society B: Biological Sciences 278:218–224.

Bernatchez, L., and C. Landry. 2003. MHC studies in nonmodel vertebrates: What have we learned about natural selection in 15 years? Journal of Evolutionary Biology 16:363–377.

Best, A. 2018. Host–pathogen coevolution in the presence of predators: fluctuating selection and ecological feedbacks. Proceedings of the Royal Society B: Biological Sciences 285:20180928.

Best, R. J., J. M. Anaya-Rojas, M. C. Leal, D. W. Schmid, O. Seehausen, and B. Matthews. 2017. Transgenerational selection driven by divergent ecological impacts of hybridizing lineages. Nature Ecology & Evolution 1:1757–1765.

Betts, A., C. Rafaluk, and K. C. King. 2016. Host and Parasite Evolution in a Tangled Bank. Trends in Parasitology 32:863–873.

Blais, J., C. Rico, C. van Oosterhout, J. Cable, G. F. Turner, and L. Bernatchez. 2007. MHC adaptive divergence between closely related and sympatric African cichlids. PLoS ONE 2:e734.

Blake, R. W., P. Y. L. Kwok, and K. H. S. Chan. 2006. Effects of two parasites, *Schistocephalus solidus* (Cestoda) and *Bunodera* spp. (Trematoda), on the escape fast-start performance of three-spined sticklebacks. Journal of Fish Biology 69:1345–1355.

Bolnick, D. I., and W. E. Stutz. 2017. Frequency dependence limits divergent evolution by favouring rare immigrants over residents. Nature 546:285–288.

Bonneaud, C., O. Chastel, P. Federici, H. Westerdahl, and G. Sorci. 2006*a*. Complex Mhc-based

mate choice in a wild passerine. Proceedings of the Royal Society B 273:1111–1116.

Bonneaud, C., J. Mazuc, G. Gonzalez, C. Haussy, O. Chastel, B. Faivre, and G. Sorci. 2003. Assessing the cost of mounting an immune response. The American Naturalist 161:367–379.

Bonneaud, C., J. Perez-Tris, P. Federici, O. Chastel, and G. Sorci. 2006*b*. Major histocompatibility alleles associated with local resistance to malaria in a passerine. Evolution 60:383–389.

Boughman, J. W. 2007. Condition-dependent expression of red colour differs between stickleback species. Journal of Evolutionary Biology 20:1577–90.

Boyce, S. M. 1979. Seasonality and Patterns of Natural Selection for Life Histories. The American Naturalist 114:569–583.

Bradshaw, W. E., and C. M. Holzapfel. 2006. Evolutionary response to rapid climate change. Science 312:1477–1478.

Brockhurst, M. A., and B. Koskella. 2013. Experimental coevolution of species interactions. Trends in Ecology and Evolution 28:367–375.

Brunner, F., J. Anaya-Roja, B. Matthews, and C. Eizaguirre. 2017. Experimental evidence that parasite drive eco-evolutionary feedbacks. Proceedings of the National Academy of Sciences 114:3678–3683.

Brunner, F. S., and C. Eizaguirre. 2016. Can environmental change affect host/parasite-mediated speciation? Zoology 119:384–394.

Buchholz, R., M. Jones Dukes, S. Hecht, and A. M. Findley. 2004. Investigating the turkey's "snood" as a morphological marker of heritable disease resistance. Journal of Animal Breeding and Genetics 121:176–185.

Buckling, A., and P. B. Rainey. 2002. Antagonistic coevolution between a bacterium and a

bacteriophage. Proceedings of the Royal Society B: Biological Sciences 931–936.

Cammen, K., J. I. Hoffman, L. A. Knapp, J. Harwood, and W. Amos. 2011. Geographic variation of the major histocompatibility complex in Eastern Atlantic grey seals (*Halichoerus grypus*). Molecular Ecology 20:740–752.

Candolin, U. 1997. Predation risk affects courtship and attractiveness of competing threespine stickleback males. Behavioral Ecology and Sociobiology 41:81–87.

Candolin, U. 1998. Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. Proceedings of the Royal Society B: Biological Sciences 265:1171–1175.

Candolin, U., and L. Vlieger. 2013. Should Attractive Males Sneak: The Trade-Off between Current and Future Offspring. PLoS ONE 8:e57992.

Carius, H.-J., T. J. Little, and D. Ebert. 2001. Genetic variation in a host–parasite association: Potential for coevolution and frequency dependent selection. Evolution 55:1146–1152.

Cattadori, I. M., D. T. Haydon, and P. J. Hudson. 2005. Parasites and climate synchronize red grouse populations. Nature 433:737–741.

Chaix, R., C. Cao, and P. Donnelly. 2008. Is mate choice in humans MHC-dependent? PLoS Genetics 4:1–5.

Chapman, T., L. F. Liddle, J. M. Kalb, M. F. Wolfner, and L. Partridge. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature 373:241–244.

Chappell, L. H. 1969. The Parasites of Three-spined stickleback *Gasterosteus aculeatus* L. from a Yorkshire Pond - I. seasonal variation of Parasite Fauna. Journal of Fish Biology 1:137–152.

Charbonnel, N., and J. Pemberton. 2005. A long-term genetic survey of an ungulate population reveals balancing selection acting on MHC through spatial and temporal fluctuations in selection. Heredity 95:377–388.

Chivers, D. P., and R. J. F. Smith. 1995. Free—living fathead minnows rapidly learn to recognize pike as predators. Journal of Fish Biology 46:949–954.

Clinchy, M., M. J. Sheriff, and L. Y. Zanette. 2013. Predator-induced stress and the ecology of fear. Functional Ecology 27:56–65.

Colegrave, N., J. S. Kotiaho, and J. L. Tomkins. 2002. Mate choice or polyandry: reconciling genetic compatability and good genes sexual selection. Evolutionary Ecology Research 4:911–917.

Colosimo, P. F., K. E. Hosemann, S. Balabhadra, G. Villarreal Jr, M. Dickson, J. Grimwood, J. Schmutz, et al. 2005. Widespread Parallel Evolution in Sticklebacks by Repeated Fixation of Ectodysplasin Alleles. Science 307:1928–1933.

Connell, S. D. 1998. Effects of predators on growth, mortality and abundance of a juvenile reef-fish: Evidence from manipulations of predator and prey abundance. Marine Ecology Progress Series 169:251–261.

Consuegra, S., and C. G. de Leaniz. 2008. MHC-mediated mate choice increases parasite resistance in salmon. Proceedings of the Royal Society B: Biological Sciences 275:1397–403.

Cook, L. M., B. S. Grant, and I. J. Saccheri. 2012. Selective bird predation on the peppered moth: the last experiment of Michael Majerus. Biology Letters 8:609–612.

Cresswell, W. 2008. Non-lethal effects of predation in birds. Ibis 150:3–17.

Crow, J. F., and M. Kimura. 1965. Evolution in Sexual and Asexual Populations. The American Naturalist 99:439–450.

Crowden, A. E., and D. M. Broom. 1980. Effects fo the eyefluke, *Diplostomum spathaceum*, on the behaviour of dace (*Leuciscus leuciscus*). Animal Behaviour 28:287–294.

Darwin, C. 1859. The Origin of Species by means of Natural Selection; or the Preservation of Favoured Races in the Struggle for Life. John Murray, London, UK.

———. 1862. On the various contrivances by which British and foreign orchids are fertilised by insects, and on the good effects of intercrossing. John Murray, London, UK.

Darwin, C. 1871. The Descent of Man, and the selection in relation to sex. Princeton University Press. Princeton, New Jersey.

De Assunção-Franco, M., J. I. Hoffman, J. Harwood, and W. Amos. 2012. MHC genotype and near-deterministic mortality in grey seals. Scientific Reports 2:1–3.

De Boer, R. J., J. A. M. Borghans, M. Van Boven, C. Keşmir, and F. J. Weissing. 2004. Heterozygote advantage fails to explain the high degree of polymorphism of the MHC. Immunogenetics 55:725–731.

De Castro, E. C. P., M. Zagrobelny, M. Z. Cardoso, and S. Bak. 2018. The arms race between heliconiine butterflies and *Passiflora* plants – new insights on an. Biological Reviews 93:555–573.

De Roode, J. C., and T. Lefèvre. 2012. Behavioral immunity in insects. Insects 3:789–820.

De Roode, J. C., R. M. Rarick, A. J. Mongue, N. M. Gerardo, and M. D. Hunter. 2011. Aphids indirectly increase virulence and transmission potential of a monarch butterfly parasite by reducing defensive chemistry of a shared food plant. Ecology Letters 14:453–461.

Decaestecker, E., S. Gaba, J. A. M. Raeymaekers, R. Stoks, L. Van Kerckhoven, D. Ebert, and L. De Meester. 2007. Host–parasite 'Red Queen' dynamics archived in pond sediment. Nature 450:870–873.

Des Roches, S., J. B. Shurin, D. Schluter, and L. J. Harmon. 2013. Ecological and evolutionary effects of stickleback on community structure. PloS ONE 8:e59644.

Dianne, L., M. J. Perrot-Minnot, A. Bauer, M. Gaillard, E. Léger, and T. Rigaud. 2011. Protection first then facilitation: A manipulative parasite modulates the vulnerability to predation of its intermediate host according to its own developmental stage. Evolution 65:2692–2698.

Ditchkoff, S. S., R. L. Lochmiller, R. E. Masters, S. R. Hoofer, and R. A. Van Den Bussche. 2001. Major-Histocompatibility-Complex-Associated Variation in Secondary Sexual Traits of White-Tailed Deer (*Odocoileus Virginianus*): Evidence for Good-Genes Advertisement. Evolution 55:616–625.

Dittmar, J., H. Janssen, A. Kuske, J. Kurtz, and J. P. Scharsack. 2014. Heat and immunity: An experimental heat wave alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). Journal of Animal Ecology 83:744–757.

Doherty, P. C., and R. M. Zinkernagel. 1975. Enhanced immunological surveillance in mice heterozygous at H-2 gene complex. Nature 256:50–52.

Doledec, S., and D. Chessel. 1994. Co-inertia analysis: an alternative method for studying species–environment relationships. Freshwater Biology 31:277–294.

Dowell, S. F. 2001. Seasonal Variation in Host Susceptibility and Cycles of Certain Infectious Diseases. Emerging Infectious Diseases 7:369–374.

Duchesne, P., M. Godbout, and L. Bernatchez. 2002. PAPA (package for the analysis of parental allocation):a computer program for simulated and real parental allocation. Molecular Ecology Notes 2:191–193.

Dufresne, F., G. J. Fitzgerald, S. Lachance, D. De Biologic, and U. Laval. 1986. Age and size-related differences in reproductive success and reproductive costs in threespine sticklebacks.

Behavioral Ecology 1:140–147.

Dunsmore, J. D., and M. L. Dubzinski. 1968. Relationship of Numbers of Nematode Parasites in Wild Rabbits, *Oryctolagus cuniculus* (L .), to Host Sex, Age, and Season. The Journal of Parasitology 54:462–474.

Dybdahl, M. F., and C. M. Lively. 1998. Host-Parasite Coevolution: Evidence for Rare Advantage and Time-Lagged Selection in a Natural Population. Evolution 52:1057–1066.

Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and Plants: A Study in Coevolution. Evolution 18:586–608.

Eizaguirre, C., and T. L. Lenz. 2010. Major histocompatibility complex polymorphism: Dynamics and consequences of parasite-mediated local adaptation in fishes. Journal of Fish Biology 77:2023–2047.

Eizaguirre, C., T. L. Lenz, M. Kalbe, and M. Milinski. 2012*a*. Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. Ecology Letters 15:723–731.

———. 2012*b*. Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. Nature Communications 3:1–6.

Eizaguirre, C., T. L. Lenz, R. D. Sommerfeld, C. Harrod, M. Kalbe, and M. Milinski. 2011. Parasite diversity, patterns of MHC II variation and olfactory based mate choice in diverging three-spined stickleback ecotypes. Evolutionary Ecology 25:605–622.

Eizaguirre, C., T. L. Lenz, A. Traulsen, and M. Milinski. 2009*a*. Speciation accelerated and stabilized by pleiotropic major histocompatibility complex immunogenes. Ecology Letters 12:5–12.

Eizaguirre, C., S. E. Yeates, T. L. Lenz, M. Kalbe, and M. Milinski. 2009*b*. MHC-based mate

choice combines good genes and maintenance of MHC polymorphism. Molecular Ecology 18:3316–3329.

Ejsmond, M. J., and J. Radwan. 2015. Red Queen Processes Drive Positive Selection on Major Histocompatibility Complex (MHC) Genes. PLoS ONE 11:e1004627.

Ekblom, R., S. A. Sæther, M. Grahn, P. Fiske, J. A. Kålås, and J. Höglund. 2004. Major histocompatibility complex variation and mate choice in a lekking bird, the great snipe (*Gallinago media*). Molecular Ecology 13:3821–3828.

Ekblom, R., S. A. Sæther, P. Jackobsson, P. Fiske, T. Sahlman, M. Grahn, J. A. Kålås, et al. 2007. Spatial pattern of MHC class II variation in the great snipe (*Gallinago media*). Molecular Ecology 16:1439–1451.

Emlen, S. T., and L. W. Oring. 1977. Ecology, Sexual Selection, and the Evolution of Mating Systems. Science 197:215–223.

Endler, J. 1980. Natural Selection on Color Patterns in *Poecilia reticulata*. Evolution 34:76–91.

Engqvist, L., N. Cordes, and K. Reinhold. 2014. Evolution of risk-taking during conspicuous mating displays. Evolution 69:395–406.

Engqvist, L., and M. Taborsky. 2016. The evolution of genetic and conditional alternative reproductive tactics. Proceedings of the Royal Society B: Biological Sciences 283:20152945.

Eutermoser, A. 1961. Schlagen Beizfalken bevorzugt kranke Krähen. Vogelwelt 82:101–104.

Ezenwa, V. O., and K. E. L. Worsley-Tonks. 2018. Social living simultaneously increases infection risk and decreases the cost of infection. Proceedings of the Royal Society B: Biological Sciences 285:20182142.

Faivre, B., A. Gregoire, M. Preault, F. Crezilly, and G. Sorci. 2003. Immune Activation Rapidly

Mirrored in a Secondary Sexual Trait. Science 300:103.

Ferrari, J., C. B. Müller, A. R. Kraaijeveld, and H. C. J. Godfray. 2001. Clonal Variation and Covariation in Aphid Resistance To Parasitoids and a Pathogen. Evolution 55:1805–1814.

Feulner, P. G. D., F. J. J. Chain, M. Panchal, Y. Huang, C. Eizaguirre, M. Kalbe, T. L. Lenz, et al. 2015. Genomics of divergence along a continuum of parapatric population differentiation. PLoS Genetics 11:1–18.

Fisher, R. A. 1930. The genetical theory of natural selection. Dover Publications, Inc., New York & Constable & Co., Ltd., London.

Fox, A., and P. J. Hudson. 2001. Parasites reduce territorial behaviour in red grouse (*Lagopus lagopus scoticus*). Ecology Letters 4:139–143.

Francova, K., and M. Ondrackova. 2013. Overwinter body condition, mortality and parasite infection in two size classes of 0+ year juvenile European bitterling *Rhodeus amarus*. Journal of Fish Biology 82:555–568.

Fraser, B. A., I. W. Ramnarine, and B. D. Neff. 2010. Temporal variation at the MHC class II*b* in wild populations of the guppy (*Poecilia reticulata*). Evolution 64:2086–2096.

Fretwell, J. R. 1972. Populations in a Seasonal Environment. Princeton University Press, Princeton, NJ.

Frickel, J., M. Sieber, and L. Becks. 2016. Eco-evolutionary dynamics in a coevolving host-virus system. Ecology Letters 19:450–459.

Frickel, J., L. Theodosiou, and L. Becks. 2017. Rapid evolution of hosts begets species diversity at the cost of intraspecific diversity. Proceedings of the National Academy of Science 114:11193–11198.

Friman, V. P., and A. Buckling. 2013. Effects of predation on real-time host-parasite coevolutionary dynamics. Ecology Letters 16:39–46.

Frischknecht, M. 1993. The breeding colouration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. Evolutionary Ecology 7:439–450.

Germain, R. N. 1994. MHC-dependent antigen processing and peptide presentation: Providing ligands for T lymphocyte activation. Cell 76:287–299.

Gibson, G. 2005. The synthesis and evolution of a supermodel. Science 307:1890–1891.

Gokhale, C. S., A. Papkou, A. Traulsen, and H. Schulenburg. 2013. Lotka-Volterra dynamics kills the Red Queen: population size fluctuations and associated stochasticity dramatically change host-parasite coevolution. BMC Evolutionary Biology 13:254.

Graham, A. L., A. D. Hayward, K. A. Watt, J. G. Pilkington, J. M. Pemberton, and D. H. Nussey. 2010. Fitness Correlates of Heritable Variation in Antibody Responsiveness in a Wild Mammal. Science 330:662–665.

Greischar, M. A., and B. Koskella. 2007. A synthesis of experimental work on parasite local adaptation. Ecology Letters 10:418–434.

Häberli, M. A., P. B. Aeschlimann, and M. Milinski. 2005. Sticklebacks benefit from closer predator inspection: an experimental test of risk assessment. Ethology Ecology & Evolution 17:249–259.

Hablützel, P. I., M. P. M. Vanhove, A. F. Grégoir, B. Hellemans, F. A. M. Volckaert, and J. A. M. Raeymaekers. 2014. Intermediate number of major histocompatibility complex class IIB length variants relates to enlarged perivisceral fat deposits in the blunt-head cichlid *Tropheus moorii*. Journal of Evolutionary Biology 27:2177–2190.

Hafer, N., and M. Milinski. 2016. Inter- and intraspecific conflicts between parasites over host manipulation. Proceedings of the Royal Society B: Biological Sciences 283:20152870.

Haldane, J. B. S. 1949. Disease and Evolution. La Ricerca Scientifica 19:68–76.

Hamilton, W. D. 1980. Sex versus Non-Sex versus Parasite. Oikos 35:282–290.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384–387.

Hammerschmidt, K., K. Koch, M. Milinski, J. C. Chubb, and G. A. Parker. 2009. When to Go: Optimization of Host Switching in Parasites with Complex Life Cycles. Evolution 63:1976–1986.

Hanek, G., and C. H. Fernando. 1978. Seasonal dynamics and spatial distribution of Urocleidus ferox Mueller, 1934, a gill parasite of Lepomis gibbosus (L.). Canadian Journal of Zoology 56:1241–1243.

Hanssen, S. A., D. Hasselquist, I. Folstad, and K. E. Erikstad. 2004. Costs of immunity: immune responsiveness reduces survival in a vertebrate. Proceedings of the Royal Society B: Biological Sciences 271:925–30.

Hart, P. J. B., and S. F. Hamrin. 1990. The role of behaviour and morphology in the selection of prey by pike. Pages 235–254 *in*Behavioural Mechanisms of Food Selection. Springer.

Hayes, C. L., S. Callander, I. Booksmythe, M. D. Jennions, and P. R. Y. Backwell. 2016. Mate choice and the operational sex ratio: an experimental test with robotic crabs. Journal of Evolutionary Biology 29:1455–1461.

Hedrick, P. W. 1994. Evolutionary Genetics of the Major Histocompatibility Complex. The American Naturalist 143:945–964.

Hedrick, P. W. 2002. Pathogen resistance and genetic variation at MHC loci. Evolution 56:1902–

1908.

Hedrick, P. W., and G. Thomson. 1983. Evidence for balancing selection at HLA. Genetics 104:449–456.

Heins, D. C., S. S. Singer, and J. A. Baker. 1999. Virulence of the cestode *Schistocephalus solidus* and reproduction in infected threespine stickleback, *Gasterosteus aculeatus*. Canadian Journal of Zoology 77:1967–1974.

Higginson, A. D., T. W. Fawcett, P. C. Trimmer, J. M. McNamara, and A. I. Houston. 2012. Generalized Optimal Risk Allocation: Foraging and Antipredator Behavior in a Fluctuating Environment. The American Naturalist 180:589–603.

Hiltunen, T., and L. Becks. 2014. Consumer co-evolution as an important component of the eco-evolutionary feedback. Nature Communications 5:1–8.

Hoffmann, J. A., F. C. Kafatos, C. A. Janeway, and R. A. B. Ezekowitz. 1999. Phylogenetic perspectives in innate immunity. Science 284:1313–1318.

Hoogland, R., D. Morris, and N. Tinbergen. 1956. The Spines of Sticklebacks (*Gasterosteus* and *Pygosteus*) as Means of Defence Against Predators (*Perca* and *Esox*). Behaviour 10:205–236.

Houde, A., and J. Endler. 1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. Science 248:1405–1408.

Howard, R. S., and C. M. Lively. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature 367:554–557.

Huang, W., A. Traulsen, B. Werner, T. Hiltunen, and L. Becks. 2017. Dynamical trade-offs arise from antagonistic coevolution and decrease intraspecific diversity. Nature Communications 8:2059.

Huang, Y., F. J. J. Chain, M. Panchal, C. Eizaguirre, M. Kalbe, T. L. Lenz, I. E. Samonte, et al.

2016. Transcriptome profiling of immune tissues reveals habitat-specific gene expression between lake and river sticklebacks. Molecular Ecology 25:943–958.

Hudson, P., A. Dobson, and D. Newborn. 1998. Prevention of population cycles by parasites removal. Science 282:2256–2258.

Hudson, P. J., and A. P. Dobson. 1992. Do Parasites make Prey Vulnerable to Predation? Red Grouse and Parasites. Journal of Animal Ecology 61:681–692.

Hudson, P. J., A. P. Dobson, and K. D. Lafferty. 2006. Is a healthy ecosystem one that is rich in parasites ? Trends in Ecology & Evolution 21:381–385.

Hughes, A. I., and M. Nei. 1988. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. Nature 336:403–405.

Hughes, N. K., J. L. Kelley, and P. B. Banks. 2012. Dangerous liaisons: The predation risks of receiving social signals. Ecology Letters 15:1326–1339.

Ilmonen, P., D. J. Penn, K. Damjanovich, L. Morrison, L. Ghotbi, and W. K. Potts. 2007. Major Histocompatibility Complex Heterozygosity Reduces Fitness in Experimentally Infected Mice. Genetics 176:2501–2508.

Ilmonen, P., T. Taarna, and D. Hasselquist. 2000. Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. Proceedings of the Royal Society B: Biological Sciences 267:665–670.

Jäger, I., C. Eizaguirre, S. W. Griffiths, M. Kalbe, C. K. Krobbach, T. B. H. Reusch, H. Schaschl, et al. 2007. Individual MHC class I and MHC class II*B* diversities are associated with male and female reproductive traits in the three-spined stickleback. Journal of Evolutionary Biology 20:2005–15.

Janeway, C. A., and R. Medzhitov. 2002. Innate Immune Recognition. Annual Review of

Immunology 20:197–216.

Janeway, C. A., P. Travers, M. Walport, and M. J. Sclomchik. 2005. Immunobiology: the immune system in health and disease. New York: Garland Science Publishing.

Jennions, M. D., A. P. Moller, M. Petrie, A. P. Mller, and S. Bernard. 2001. Sexually Selected Traits and Adult Survival: A Meta-Analysis. Quarterly Review of Biology 76:3–36.

Johnson, J. B., and A. L. Basolo. 2003. Predator exposure alters female mate choice in the green swordtail. Behavioral Ecology 14:619–625.

Johnson, P. T. J., and J. T. Hoverman. 2012. Parasite diversity and coinfection determine pathogen infection success and host fitness. Proceedings of the National Academy of Sciences 109:9006–9011.

Johnson, S., and U. Candolin. 2017. Predation cost of a sexual signal in the threespine stickleback. Behavioral Ecology 8:1160–1165.

Jokela, J., M. F. Dybdahl, and C. M. Lively. 2009. The Maintenance of Sex, Clonal Dynamics, and Host-Parasite Coevolution in a Mixed Population of Sexual and Asexual Snails. The American Naturalist 174:S43–S53.

Jordan, W. C., and M. W. Bruford. 1998. New perspectives on mate choice and the MHC. Heredity 81:127.

Kalbe, M., C. Eizaguirre, I. Dankert, T. B. H. Reusch, R. D. Sommerfeld, K. M. Wegner, and M. Milinski. 2009. Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. Proceedings of the Royal Society B: Biological Sciences 276:925–934.

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

Kalbe, M., K. M. Wegner, and T. B. H. Reusch. 2002. Dispersion patterns of parasites in 0+ year three-spined sticklebacks: A cross population comparison. Journal of Fish Biology 60:1529–1542.

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

Karlsson, B. 1998. Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. Ecology 79:2931–2940.

Karvonen, A., K. Lucek, D. a. Marques, and O. Seehausen. 2015. Divergent Macroparasite Infections in Parapatric Swiss Lake-Stream Pairs of Threespine Stickleback (Gasterosteus aculeatus). PLoS ONE 10:e0130579.

Kaufmann, J., C. Eizaguirre, M. Milinski, and T. L. Lenz. 2015. The contribution of post-copulatory mechanisms to incipient ecological speciation in sticklebacks. Biology Letters 11:20140933.

Kaufmann, J., T. L. Lenz, M. Kalbe, M. Milinski, and C. Eizaguirre. 2017. A field reciprocal transplant experiment reveals asymmetric costs of migration between lake and river ecotypes of three-spined sticklebacks (*Gasterosteus aculeatus*). Journal of Evolutionary Biology 30:938–950.

Kawahara, R., and M. Nishida. 2006. Multiple occurrences of spiggin genes in sticklebacks. Gene 373:58–66.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters 7:1225–1241.

Kay, K. M., P. A. Reeves, R. G. Olmstead, and D. W. Schemske. 2005. Rapid speciation and the evolution of hummingbird pollination in neotropical *Costus* subgenus *Costus* (Costaceae): Evidence from nrDNA its and ETS sequences. American Journal of Botany 92:1899–1910.

Kimura, M. 1968. Evolutionary Rate at the Molecular Level. Nature 217:624–626.

King, K. C., and C. M. Lively. 2012. Does genetic diversity limit disease spread in natural host populations. Heredity 109:199–203.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. Evolution 36:1–12.

Klein, J., and F. Figueroa. 1986. Evolution of the major histocompatibility complex. Critical reviews in immunology 6:295—386.

Klein, J., A. Sato, and N. Nikolaidis. 2007. MHC, TSP, and the Origin of Species: From Immunogenetics to Evolutionary Genetics. Annual Review of Genetics 41:281–304.

Klein, L., B. Kyewski, P. M. Allen, and K. A. Hogquist. 2014. Positive and negative selection of the T cell repertoire : what thymocytes see (and don't see). Nature Reviews 14:377–391.

Kloch, A., W. Babik, A. Bajer, E. Si??ski, and J. Radwan. 2010. Effects of an MHC-DRB genotype and allele number on the load of gut parasites in the bank vole *Myodes glareolus*. Molecular Ecology 19:255–265.

Knowles, S. C. L., A. Fenton, O. L. Petchey, T. R. Jones, R. Barber, and A. B. Pedersen. 2013. Stability of within-host-parasite communities in a wild mammal system. Proceedings of the Royal Society B: Biological Sciences 280:20130598.

Kokko, H. 2001. Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecology Letters 4:322–326.

Kokko, H. 2017. Give one species the task to come up with a theory that spans them all: what good can come out of that? Proceedings of the Royal Society B: Biological Sciences 284:20171652.

Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. Proceedings of the Royal Society B: Biological Sciences 270:653–664.

Kondrashov, A. S. 1988. Deleterious mutations and the evolution of sexual reproduction. Nature 336:435–440.

Koskella, B., and C. M. Lively. 2009. Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. Evolution 63:2213–2221.

Kotiaho, J., R. V Alatalo, J. Mappes, S. Parri, and A. Rivero. 1998. Male mating success and risk predation in a wolf spider: a balance between sexual and natural selection? Journal of Animal Ecology 67:287–291.

Koyasu, S., and K. Moro. 2011. Type 2 innate immune responses and the natural helper cell. Immunology 132:475–481.

Krebs, C. J., R. Boonstra, S. Boutin, and A. R. E. Sinclair. 2001. What Drives the 10-year Cycle of Snowshoe Hares? BioScience 51:25–35.

Krueger, D. A., and S. I. Dodson. 1981. Embryological induction and predation ecology in *Daphnia pulex*. Limnology and Oceanography 26:219–223.

Kuijper, B., I. Pen, and F. J. Weissing. 2012. A Guide to Sexual Selection Theory. Annual Review of Ecology, Evolution, and Systematics 43:287–311.

Külling, D., and M. Milinski. 1992. Size-dependent predation risk and partner quality in predator inspection of sticklebacks. Animal Behaviour 44:949–955.

Kuris, A. M., R. F. Hechinger, J. C. Shaw, K. L. Whitney, L. Aguirre-Macedo, C. A. Boch, A. P. Dobson, et al. 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature 454:515–518.

Kurtz, J., and K. Franz. 2003. Evidence for memory in invertebrate immunity. Nature 425:37–38.

Kurtz, J., M. Kalbe, P. B. Aeschlimann, M. A. Häberli, K. M. Wegner, T. B. H. Reusch, and M. Milinski. 2004. Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. Proceedings of the Royal Society B: Biological Sciences 271:197–204.

Lafferty, K. D., and A. K. Morris. 1996. Altered Behavior of Parasitized Killifish Increases Susceptibility to Predation by Bird Final Hosts. Ecology 77:1390–1397.

Landry, C., D. Garant, P. Duchesne, and L. Bernatchez. 2001. "Good genes as heterozygosity": the major histocompatibility complex and mate choice in Atlantic salmon (Salmo salar). Proceedings of the Royal Society B: Biological Sciences 268:1279–85.

Largiader, C. R., V. Fries, and T. C. M. Bakker. 2001. Genetic analysis of sneaking and egg-thievery in a natural population of the three-spined stickleback (*Gasterosteus aculeatus* L.). Heredity 86:459–468.

Lawlor, D. a, J. Zemmour, P. D. Ennis, and P. Parham. 1990. Evolution of class-I MHC genes and proteins: from natural selection to thymic selection. Annual Review of Immunology 8:23–63.

Lazzaro, B. P., and T. J. Little. 2009. Immunity in a variable world. Philosophical transactions of the Royal Society of London. Series B: Biological Sciences 364:15–26.

Leinders-Zufall, T., P. Brennan, P. Widmayer, C. S. Prashanth, A. Maul-Pavicic, M. Jaeger, L. Xiao-Hong, et al. 2004. MHC Class I Peptides as Chemosensory Signals in the Vomeronasal Organ. Science 306:1033–1037.

Lenz, T. L., C. Eizaguirre, S. Becker, and T. B. H. Reusch. 2009*a*. RSCA genotyping of MHC for high-throughput evolutionary studies in the model organism three-spined stickleback Gasterosteus aculeatus. BMC Evolutionary Biology 9:57.

Lenz, T. L., C. Eizaguirre, B. Rotter, M. Kalbe, and M. Milinski. 2013. Exploring local immunological adaptation of two stickleback ecotypes by experimental infection and transcriptome-

wide digital gene expression analysis. Molecular Ecology 22:774–786.

Lenz, T. L., C. Eizaguirre, J. P. Scharsack, M. Kalbe, and M. Milinski. 2009*b*. 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 75:2122–2142.

Lenz, T. L., C. Eizaguirre, J. P. Scharsack, M. Kalbe, and M. Milinski. 2009*c*. 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 75:2122–2142.

Lenz, T. L., C. Eizaguirre, J. P. Scharsack, M. Kalbe, and M. Milinski. 2009*d*. 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 75:2122–2142.

Lenz, T. L., N. Hafer, I. E. Samonte, S. E. Yeates, and M. Milinski. 2018. Cryptic haplotype-specific gamete selection yields offspring with optimal MHC immune genes. Evolution 72:2478–2490.

Lenz, T. L., K. Wells, M. Pfeiffer, and S. Sommer. 2009*e*. Diverse MHC IIB allele repertoire increases parasite resistance and body condition in the Long-tailed giant rat (*Leopoldamys sabanus*). BMC Evolutionary Biology 9:269.

Lively. 1987. Evidence from a New Zealand snail for the maintainence of sex by parasitism. Nature 8–10.

Lively, C. M. 2010*a*. The Effect of Host Genetic Diversity on Disease Spread. The American Naturalist 175:E149–E152.

Lively, C. M. 2010*b*. A review of red queen models for the persistence of obligate sexual reproduction. Journal of Heredity 101:13–20.

Lively, C. M. 2010*c*. The Effect of Host Genetic Diversity on Disease Spread. The American Naturalist 175:E149–E152.

Lively, C. M., C. Craddock, and R. C. Vrijenhoek. 1990. Red Queen hypothesis supported by parasitism in sexual and clonal fish. Nature 344:864–866.

Lively, C. M., S. G. Johnson, L. F. Delph, and K. Clay. 1995. Thinning reduces the effect of rust infection on jewelweed (*Impatiens capensis*). Ecology 76:1859–1862.

Lochmiller, R. L., and C. Deerenberg. 2000. Trade-Offs in Evolutionary Immunology: Just What Is the Cost of Immunity? Oikos 88:87–98.

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences 100:567–571.

Lohm, J., M. Grahn, A. Langefors, Ø. Andersen, A. Storset, and T. von Schantz. 2002. Experimental evidence for major histocompatibility complex-allele-specific resistance to a bacterial infection. Proceedings of the Royal Society B: Biological Sciences 269:2029–2033.

Lopez-Sepulcre, A., and H. Kokko. 2005. Territorial defense, territory size, and population regulation. The American Naturalist 166:317–329.

Lotz, J. M., A. Bush, and W. F. Font. 1995. Recruitment-Driven, Spatially Discontinuous Communities: A Null Model for Transferred Patterns in Target Communities of Intestinal Helminths. The Journal of Parasitology 81:12–24.

Lucek, K., A. Sivasundar, and O. Seehausen. 2012. Evidence of adaptive evolutionary divergence

during biological invasion. PLoS ONE 7:e49377.

Lumley, A. J., Ł. Michalczyk, J. J. N. Kitson, L. G. Spurgin, C. A. Morrison, J. L. Godwin, M. E. Dickinson, et al. 2015. Sexual selection protects against extinction. Nature 522:470–473.

Maan, M. E., and O. Seehausen. 2011. Ecology, sexual selection and speciation. Ecology Letters 14:591–602.

MacColl, A. D. C. 2009. Parasite burdens differ between sympatric three-spined stickleback species. Ecography 32:153–160.

MacNab, V., and I. Barber. 2012. Some (worms) like it hot: Fish parasites grow faster in warmer water, and alter host thermal preferences. Global Change Biology 18:1540–1548.

Madsen, T., and B. Ujvari. 2006. MHC class I variation associates with parasite resistance and longevity in tropical pythons. Journal of Evolutionary Biology 19:1973–1978.

March, W. A., and D. M. Watson. 2010. The contribution of mistletoes to nutrient returns: Evidence for a critical role in nutrient cycling. Australian Ecology 35:713–721.

Marques, D. A., K. Lucek, J. I. Meier, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen. 2016. Genomics of rapid incipient speciation in sympatric Threespine Stickleback. PLoS Genetics 12:1–34.

Martin II, L. B., A. Scheuerlein, and M. Wikelski. 2003. Immune activity elevates energy expenditure of house sparrows: A link between direct and indirect costs? Proceedings of the Royal Society B: Biological Sciences 270:153–158.

Matthews, B., T. Aebischer, K. E. Sullam, B. Lundsgaard-Hansen, and O. Seehausen. 2016. Experimental evidence of an eco-evolutionary feedback during adaptive divergence. Current Biology 26:1–7.

Maynard Smith, J. 1978. The evolution of sex. No. 574.1 S5. Cambridge: Cambridge University Press.

Mays, H. L., and G. E. Hill. 2004. Choosing mates: good genes versus genes that are a good fit. Trends in Ecology & Evolution 19:554–559.

Medzhitov, R., and C. A. Janeway. 1997. Innate immunity: The virtues of a nonclonal system of recognition. Cell 91:295–298.

Metz, M. C., D. J. Emlen, D. R. Stahler, D. R. MacNulty, D. W. Smith, and M. Hebblewhite. 2018. Predation shapes the evolutionary traits of cervid weapons. Nature Ecology & Evolution 2:1619–1625.

Meyer, J., and R. Kassen. 2007. The effects of competition and predation on diversification in a model adaptive radiation. Nature 446:432–435.

Milinski, M. 1984. Parasites determine a predator's optimal feeding strategy. Behavioral Ecology and Sociobiology 15:35–37.

———. 1985. Risk of Predation of Parasitized Sticklebacks (*Gasterosteus Aculeatus* L.) Under Competition for Food. Behaviour 93:203–216.

———. 1986. Constraints placed by predators on feeding behaviour. Pages 236–252 *in*The behaviour of teleost fishes. Edited by: Pitcher T.J.

———. 1987. TIT FOR TAT in sticklebacks and the evolution of cooperation. Nature 325:433–435.

———. 1990. Parasites and host decision-making. Pages 95–116 *in* C. J. Barnard and J. M. Behnke, eds. Parasitism and Host Behaviour. Ed.: Barnard, C. J.; Behnke, J. M.

Milinski, M. 1993. Predation risk and foraging behaviour. Pages 285–305 *in*T.J. Pitcher (ed.)

Behaviour of Teleost Fishes. 2nd Editon, Chapman & Hall, London.

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

———. 2014. Arms races, ornaments and fragrant genes: The dilemma of mate choice in fishes. Neuroscience and Biobehavioral Reviews 46:6–11.

———. 2015. Mate Choice Optimizes Offspring MHC Genetics and Drives Sexual Reproduction. Immunogenetics 1:1–6.

Milinski, M., and T. C. M. Bakker. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. Nature 344:330–333.

Milinski, M., and T. C. M. Bakker. 1992. Costs Influence Sequential Mate Choice in Sticklebacks, Gasterosteus aculeatus. Proceedings of the Royal Society B: Biological Sciences 250:229–233.

Milinski, M., I. Croy, T. Hummel, and T. Boehm. 2013. Major histocompatibility complex peptide ligands as olfactory cues in human body odour assessment. Proceedings of the Royal Society B: Biological Sciences 280:20122889.

Milinski, M., S. W. Griffiths, T. B. H. Reusch, and T. Boehm. 2010. Costly major histocompatibility complex signals produced only by reproductively active males, but not females, must be validated by a “maleness signal” in three-spined sticklebacks. Proceedings of the Royal Society B: Biological Sciences 277:391–398.

Milinski, M., S. Griffiths, K. M. Wegner, T. B. H. Reusch, A. Haas-Assenbaum, and T. Boehm. 2005. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. Proceedings of the National Academy of Sciences 102:4414–4418.

Milinski, M., and R. Heller. 1978. Influence of a predator on the optimal foraging behaviour of

Sticklebacks (*Gasterosteus aculeatus* L.). Nature 275:642–644.

Mitchell, S. E., E. S. Rogers, T. J. Little, A. F. Read, S. Url, and T. O. M. J. Little. 2005. Host-Parasite and Genotype-by-Environment Interactions : Temperature Modifies Potential for Selection by a Sterilizing Pathogen. Evolution 59:70–80.

Møller, A., and M. Jennions. 2001. How important are direct fitness benefits of sexual selection? Naturwissenschaften 88:401–415.

Møller, A. P. 1993. Ectoparasites Increase the Cost of Reproduction in their Hosts. Journal of Animal Ecology 62:309–322.

Morran, L. T., O. G. Schmidt, I. A. Gelarden, R. C. Parrish II, and C. M. Lively. 2011. Running with the Red Queen: Host-parasite coevolution selects for biparental sex. Science 333:216–218.

Moya, A., E. C. Holmes, and F. González-Candelas. 2004. The population genetics and evolutionary epidemiology of RNA viruses. Nature Reviews Microbiology 2:279–288.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 1:2–9.

Nagar, A. El, and A. D. C. MacColl. 2016. Parasites contribute to ecologically dependent postmating isolation in the adaptive radiation of three-spined stickleback. Proceedings of the Royal Society B 283:20160691.

Navarro, C., F. De Lope, A. Marzal, and A. P. Møller. 2004. Predation risk, host immune response, and parasitism. Behavioral Ecology 15:629–635.

Neff, B. D., and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14:19–38.

Nowak, M. A., K. Tarczy-Hornoch, and J. M. Austyn. 1992. The optimal number of major

histocompatibility complex molecules in an individual. Proceedings of the National Academy of Science 89:10896–10899.

Nuismer, S. L., R. Gomulkiewicz, and M. T. Morgan. 2003. Coevolution in temporally variable environments. The American Naturalist 162:195–204.

Nuismer, S. L., S. P. Otto, and F. Blanquart. 2008. When do host-parasite interactions drive the evolution of non-random mating? Ecology Letters 11:937–946.

Nurmonsky, D., M. V Nurminskaya, D. De Aguiar, and D. L. Hartl. 1998. Selective sweep of a newly evolved sperm-specific gene in *Drosophila*. Nature 396:572–575.

Oliver, M. K., S. Telfer, and S. B. Piertney. 2009. Major histocompatibility complex (MHC) heterozygote superiority to natural multi-parasite infections in the water vole (*Arvicola terrestris*). Proceedings of the Royal Society B 276:1119–1128.

Packer, C. 1983. Sexual dimorphism: The horns of African antelopes. Science 221:1191–1193.

Palm, N. W., and R. Medzhitov. 2009. Pattern recognition receptors and control of adaptive immunity. Immunological Reviews 227:221–233.

Papkou, A., C. S. Gokhale, A. Traulsen, and H. Schulenburg. 2016. Host–parasite coevolution: why changing population size matters. Zoology 119:330–338.

Paterson, S., K. Wilson, and J. M. Pemberton. 1998. Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population. Proceedings of the National Academy of Sciences 95:3714–3719.

Peichel, C. L., and J. W. Boughman. 2003. Sticklebacks. Current Biology 13:R942–R943.

Peichel, C. L., K. S. Nereng, K. a Ohgi, B. L. Cole, P. F. Colosimo, C. a Buerkle, D. Schluter, et al. 2001. The genetic architecture of divergence between threespine stickleback species. Nature

414:901–905.

Penn, D. J., K. Damjanovich, and W. K. Potts. 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. Proceedings of the National Academy of Science 99:11260–11264.

Penn, D. J., and W. K. Potts. 1999. The evolution of mating preferences and major histocompatibility complex genes. The American Naturalist 153:145–164.

Pennycuick, L. 1971. Seasonal variations in the parasite infections in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L. Parasitology 63:373–388.

Pérez-Jvostov, F., A. P. Hendry, G. F. Fussmann, and M. E. Scott. 2012. Are host-parasite interactions influenced by adaptation to predators? A test with guppies and *Gyrodactylus* in experimental stream channels. Oecologia 170:77–88.

Phillips, K. P., J. Cable, R. S. Mohammed, M. Herdegen-Radwan, J. Raubic, K. J. Przesmycka, C. van Oosterhout, et al. 2018. Immunogenetic novelty confers a selective advantage in host–pathogen coevolution. Proceedings of the National Academy of Sciences 115:1552–1557.

Piertney, S. B., and M. K. Oliver. 2006. The evolutionary ecology of the major histocompatibility complex. Heredity 96:7–21.

Pitcher, T. E., and B. D. Neff. 2006. MHC class IIB alleles contribute to both additive and nonadditive genetic effects on survival in Chinook salmon. Molecular Ecology 15:2357–2365.

Potts, W. K., C. J. Manning, and E. K. Wakeland. 1991. Mating patterns in seminatural populations of mice influenced by MHC genotype. Nature 352:619–621.

Poulin, R. 2007. Evolutionary ecology of parasites: (Second edition). Princeton University Press.

Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E.

La Marca, et al. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161–167.

Puurtinen, M., T. Ketola, and J. S. Kotiaho. 2009. The Good-Genes and Compatible-Genes Benefits of Mate Choice. The American Naturalist 174:741–752.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Raberg, L., D. Sim, and A. F. Read. 2007. Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. Science 318:812–814.

Rauch, G., M. Kalbe, and T. B. H. Reusch. 2006. One day is enough: Rapid and specific host-parasite interactions in a stickleback-trematode system. Biology Letters 2:382–384.

Relle, M., and A. Schwarting. 2012. Role of MHC-linked susceptibility genes in the pathogenesis of human and murine lupus. Clinical and Developmental Immunology 2012.

Reusch, T. B. H., M. A. Häberli, P. B. Aeschlimann, and M. Milinski. 2001*a*. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature 414:300–302.

Reusch, T. B. H., and A. Langefors. 2005. Inter- and intralocus recombination drive MHC class IIB gene diversification in a teleost, the three-spined stickleback *Gasterosteus aculeatus*. Journal of Molecular Evolution 61:531–541.

Reusch, T. B. H., H. Schaschl, and K. M. Wegner. 2004. Recent duplication and inter-locus gene conversion in major histocompatibility class II genes in a teleost, the three-spined stickleback. Immunogenetics 56:427–437.

Reusch, T. B. H., K. M. Wegner, and M. Kalbe. 2001*b*. Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage

and geographical proximity. Molecular Ecology 10:2435–2445.

Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346:183–187.

Richner, H., and F. Tripet. 1999. Ectoparasitism and the Trade-Off between Current and Future Reproduction. Oikos 86:535–538.

Ripple, W. J., E. J. Larsen, R. a Renkin, and D. W. Smith. 2001. Trophic cascades among wolves, elk and aspen on Yellowstone National Park's northern range. Biological Conservation 102:227–234.

Roberts, S. C., and L. M. Gosling. 2003. Genetic similarity and quality interact in mate choice decisions by female mice. Nature Genetics 35:103–106.

Rowan, R., and N. Knowlton. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proceedings of the National Academy of Science 92:2850–2853.

Ruell, E. W., C. A. Handelsman, C. L. Hawkins, H. R. Sofaer, C. K. Ghalambor, and L. Angeloni. 2013. Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. Proceedings of the Royal Society B: Biological Sciences 280:20122019.

Ryan, M. J., and M. E. Cummings. 2013. Perceptual Biases and Mate Choice. Annual Review of Ecology, Evolution, and Systematics 44:437–459.

Saleem, M., I. Fetzer, C. F. Dormann, H. Harms, and A. Chatzinotas. 2012. Predator richness increases the effect of prey diversity on prey yield. Nature Communications 3:1305.

Sato, A., F. Figueroa, C. O'hUigin, N. Steck, and J. Klein. 1998. Cloning of major histocompatibility complex (Mhc) genes from threespine stickleback, *Gasterosteus aculeatus*. Molecular Marine Biology and Biotechnology 7:221–231.

Scharsack, J. P., M. Kalbe, C. Harrod, and G. Rauch. 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.

Schluter, D. 1993. Adaptive radiation in sticklebacks: Size, shape, and habitat use efficiency. Ecology 74:699–709.

———. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology 76:82–90.

Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. Proceedings of the Royal Society of London B: Biological Sciences 270:357–366.

———. 2011. Evolutionary Parasitology. The integrated study of infections,immunology, ecology and genetics. Oxford University Press, Oxford. UK.

Schmid, D. W., M. D. Mcgee, R. J. Best, O. Seehausen, and B. Matthews. 2019. Rapid Divergence of Predator Functional Traits Affects Prey Composition in Aquatic Communities. The American Naturalist 193:331–345.

Schneeberger, K., A. Courtiol, G. A. Czirjak, and C. C. Voigt. 2014. Immune Profile Predicts Survival and Reflects Senescence in a Small, Long-Lived Mammal, the Greater Sac-Winged Bat (*Saccopteryx bilineata*). PLoS ONE 9:e108268.

Schrader, M. S., E. L. Walters, F. C. James, and E. C. Greiner. 2003. Seasonal Prevalence of a Haematozoan Parasite of Red-Bellied Woodpeckers (*Melanerpes carolinus*) and Its Association with Host Condition and Overwinter Survival. The Auk 120:130–137.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology - costly parasite defenses and trade-offs in evolutionary ecology. Trends in Ecology & Evolution 11:317–321.

Sherborne, A. L., M. D. Thom, S. Paterson, F. Jury, W. E. R. Ollier, P. Stockley, R. J. Beynon, et al. 2007. The Genetic Basis of Inbreeding Avoidance in House Mice. Current Biology 17:2061–2066.

Sheriff, M. J., C. J. Krebs, R. Boonstra, M. J. Sheriff, C. J. Krebs, and R. Boonstra. 2011. From process to pattern : how fluctuating predation risk impacts the stress axis of snowshoe hares during the 10-year cycle. Oecologia 166:593–605.

Spurgin, L. G., and D. S. Richardson. 2010. How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. Proceedings of the Royal Society B: Biological Sciences 277:979–88.

Steck, N., C. Wedekind, and M. Milinski. 1999. No sibling odor preference in juvenile three-spined sticklebacks. Behavioral Ecology 10:493–497.

Stet, R. J. M., C. P. Kruiswijk, and B. Dixon. 2003. Major Histocompatibility Lineages and Immune Gene Function in Teleost Fishes: The Road Not Taken. Critical Reviews in Immunology 23:473–488.

Stewart, A., P. I. Hablützel, H. V Watson, M. Brown, I. M. Friberg, J. Cable, and J. A. Jackson. 2018. Physical cues controlling seasonal immune allocation in a natural Piscine Model. Frontiers in Immunology 9:1–15.

Stewart, A., J. Jackson, I. Barber, C. Eizaguirre, R. Paterson, P. van West, C. Williams, et al. 2017. Hook, Line and Infection: A Guide to Culturing Parasites, Establishing Infections and Assessing Immune Responses in the Three-Spined Stickleback. Advances in Parasitology 98:39–109.

Stiebens, V. A., S. E. Merino, F. J. J. Chain, and C. Eizaguirre. 2013. Evolution of MHC class i genes in the endangered loggerhead sea turtle (Caretta caretta) revealed by 454 amplicon sequencing. BMC Evolutionary Biology 13:1–11.

Stromberg, B. E. 1997. Environmental factors influencing transmission. Veterinary Parasitology 72:247–264.

Svensson, E. I. 2019. Eco - evolutionary dynamics of sexual selection and sexual conflict. Functional Ecology 33:60–72.

Svensson, E. I., and T. P. Gosden. 2007. Contemporary evolution of secondary sexual traits in the wild. Functional Ecology 21:422–433.

Takahata, N., and M. Nei. 1990. Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. Genetics 124:967–978.

Takeda, K., and S. Akira. 2005. Toll-like receptors in innate immunity. International immunology 17:1–14.

Ter Hofstede, H. M., S. Schöneich, T. Robillard, and B. Hedwig. 2015. Evolution of a Communication System by Sensory Exploitation of Startle Behavior. Current Biology 25:3245–3252.

Thomas, F., F. Renaud, T. de Meeus, and R. Poulin. 1998. Manipulation of host behaviour by parasites: Ecosystem engineering in the intertidal zone? Proceedings of the Royal Society B: Biological Sciences 265:1091–1096.

Thompson, J. N. 1994. The coevolutionary process. University of Chicago Press.

Thompson, J. N. 2013. Relentless Evolution. University of Chicago Press.

Tinbergen, N. 1951. The study of instinct. Oxford, UK: Clarendon Press. 22.

Torchin, M. E., K. D. Lafferty, A. P. Dobson, V. J. McKenzie, and A. M. Kuris. 2003. Introduced species and their missing parasites. Nature 421:628–630.

Tregenza, T., and N. Wedell. 2000. Genetic compatibility, mate choice and patterns of parentage:

Invited review. Molecular Ecology 9:1013–1027.

Trivers, R. 1985. Social Evolution. Menlo Park, CA: Banjamin Cummings.

Troost, T. A., J. A. van Dam, B. W. Kooi, and E. Tuenter. 2009. Seasonality, Climate Cycles and Body Size Evolution. Mathematical Modelling of Natural Phenomena 4:135–155.

Tschirren, B., M. Andersson, K. Scherman, H. Westerdahl, P. R. E. Mittl, and L. Råberg. 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 280:20130364.

Tschirren, B., and H. Richner. 2006. Parasites shape the optimal investment in immunity. Proceedings of the Royal Society B: Biological Sciences 273:1773–1777.

Van Schaik, J., and G. Kerth. 2017. Host social organization and mating system shape parasite transmission opportunities in three European bat species. Parasitology Research 116:589–599.

Van Valen, L. 1973. A new evolutionary law. Evolutionary Theory 1:1–30.

Wacker, S., K. Mobley, E. Forsgren, L. C. Myhre, K. de Jong, and T. Amundsen. 2013. Operational Sex Ratio But Not Density Affects Sexual Selection In A Fish. Evolution 67:1937–1949.

Wakeland, E., S. Boehme, J. She, C. Lu, R. McIndoe, I. Cheng, Y. Ye, et al. 1990. Ancestral polymorphisms of Mhc class-II genes—divergent allele advantage. Immunologic Research 9:115–122.

Watters, G. T., and S. H. O'Dee. 2000. Glochidial release as a function of water temperature: Beyond bradyticty and tachyticty. Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium, 1998 135–140.

Weber, J. N., N. C. Steinel, K. C. Shim, and D. I. Bolnick. 2017. Recent evolution of extreme cestode growth suppression by a vertebrate host. Proceedings of the National Academy of Sciences

114:6575–6580.

Wedekind, C., T. Seebeck, F. Bettens, and A. Paepke. 1995. MHC-dependent mate preference in humans. Proceedings of the Royal Society B: Biological Sciences 260:245–249.

Wegner, K. M., and C. Eizaguirre. 2012. New(t)s and views from hybridizing MHC genes: Introgression rather than trans-species polymorphism may shape allelic repertoires. Molecular Ecology 21:779–781.

Wegner, K. M., M. Kalbe, J. Kurtz, T. Reusch, and M. Milinski. 2003*a*. Parasite Selection for Immunogenetic Optimality. Science 301:1343–1343.

Wegner, K. M., M. Kalbe, M. Milinski, and T. B. Reusch. 2008. Mortality selection during the 2003 European heat wave in three-spined sticklebacks: effects of parasites and MHC genotype. BMC Evolutionary Biology 8:1–12.

Wegner, K. M., T. B. H. Reusch, and M. Kalbe. 2003*b*. Multiple parasites are driving major histocompatibility complex polymorphism in the wild. Journal of Evolutionary Biology 16:224–232.

Westerdahl, H., B. Hansson, S. Bensch, and D. Hasselquist. 2004. Between-year variation of MHC allele frequencies in great reed warblers: Selection or drift? Journal of Evolutionary Biology 17:485–492.

Wetton, J. H., R. E. Carter, D. T. Parkin, and D. Walters. 1987. Demographic study of a wild house sparrow populationby DNA fingerprinting. Nature 327:147–149.

Windsor, D. A. 1998. Most of the species on Earth are parasites. International Journal for Parasitology 28:1939–1941.

Woelfing, B., A. Traulsen, M. Milinski, and T. Boehm. 2009. Does intra-individual major

histocompatibility complex diversity keep a golden mean? Philosophical Transactions of the Royal Society B: Biological Sciences 364:117–128.

Wolinska, J., and K. C. King. 2009. Environment can alter selection in host-parasite interactions. Trends in Parasitology 25:236–244.

Woolhouse, M. E. J., J. P. Webster, E. Domingo, B. Charlesworth, and B. R. Levin. 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts 32:569–577.

Wooster, D. 1994. Predator Impacts on Stream Benthic Prey. Oecologia 99:7–15.

Wootton, R. J. 1976. The biology of the sticklebacks. London, Academic Press.

Yamazaki, B. Y. K., E. A. Boyse, H. T. Thaler, B. J. Mathieson, J. Abbott, J. Boyse, Z. A. Zayas, et al. 1976. Control of mating preferences in mice by genes of the Major Histocompatibility Complex. The Journal of Experimental Medicine 144:1324–1335.

Young, R. E., and A. D. C. MacColl. 2017. Spatial and temporal variation in macroparasite communities of three-spined stickleback. Parasitology 144:436–449.

Zahavi, A. 1975. Mate Selection - Selection for a Handicap. Journal of Theoretical Biology 53:205–214.

Zanette, L. Y., A. F. White, M. C. Allen, and M. Clinchy. 2011. Perceived Predation Risk Reduces the Number of Offspring Songbirds Produce per Year. Science 334:1398–1401.

Zuk, M., and G. Kolluru. 1998. Exploitation of sexual signals by predators and parasitoids. The Quaterly Review of Biology 73:3–22.

# SUPPLEMENTARY MATERIAL

**Appendix 1. Sequence of those alleles not deposited at the NCBI**

**No20**

GAGTACATCAGGTCTTATTACTACAGCAAGTTAGAATACACGAGGTTCAGCAGCTCAGTGG
GGAAGTATGTCGGCTTCACTGAGTACGGAGTGAAGAACGCTGCAGCCTGGAACAACAACC
CTTCAATTCTGAGTAGAGCGAAGGCTCATAAGGAGGCTTACTGTCTGCACAACATCCAGAT
CGATTACAACAATATGCTGACTAAGTCCG

**No40**

GAGTTCATCAGGTCTTTTTACTACAACAAGTTAGAACTCACGAGGTTCAGCAGCTCAGTGG
GGAAGTTTGTCGGCTTCACTGAGTACGGAGTGAGGAACGCTGAATACTGGAACAACGACGC
TTCACTTCTGAGTGCTATGAAGGCTCAGAAGGAGGTTTACTGTCTGAACAACGTCCCGGTCT
ATTACAGAGCTGCTCTGACTAAGTCCGGTGAGT

**No41**

GAGTTCATCAGGTCTGTTTACTACAACAAGTTAGAATTCACGAGGTTCAGCAGCTCAGTGG
GGAAGTTTGTCGGCTTCACTGAGTACGGAGTGAGGAACGCTGAATACTGGAACAAAGACG
CTTCATTTCTGAGTGCTATGAAGGCTCAGAAGGAGGGTTACTGTCTGCACAACATCCAGAA
CTGGTACAACAATATGCTGACTAAGTCCGGTGAGT

**No50**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTTCATCAGGTCTGTTTACTACAACAAGTT
AGAATTCACGAGGTTCAGCAGCTCAGTGGGGAAGTTTGTCGGCTTCACTGAGTACGGAGTG
AAGAACGCTGAATACTGGAACAACGACCCTTCAATTCTGAGTAGAGCGAAGGCTCAGAAG
GAGGGTTACTGTCTGCACAACATCCAGAACTGGTACAACAATATGCTGACTAAGTCCGGTG
AGT

**No51**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTTCATCGACTCGTATTACTACAACAAGTT
AGAATACACGAGGTTCAGCAGCTCAGTGGGGAAGTTTGTCGGCTTCACTGAACGCGGAGTG
AGGAACGCTGAATACTGGAACAACGACCCTTCACTTCTGAGTGCTATGAAGGCTCAGAAGG
AGGCCGTCTGTCTGCACAACATCCAGATCAAATATGACAATGCTCTGACTAAGTCCGGTGA
GT

**No54**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTACATCAGGTCTTCTTACTTCAACAAGA
AAGAAGACACGAGGTTCAGCAGCTCAGTGGGGAAGTTTGTCGGCTTCACTGAACAAGGAG
TGAAGTTCGCTGCAGCCTGGAACAACAACCCTTCATATCTGAGTGCTATGAAGGCTCAGAA
GGAGGCCGTCTGTCTGAACCACATCCAGATCGAGTACAACAATATGCTGACTAAGTCCGGT
GAGT

**No55**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTTCATCAGGTCTTCTTACTTCAACAAGTT
AGAATACACGAGGTTCAGCAGCTCAGTGGGGAAGTTTGTCGGCTTCACTGAACAAGGAGTG
AGGAACGCTGAATACTGGAACAACGACGCTTCACTTCTGAGTGCTATGAAGGCTCAGAAGG
AGGTTTACTGTCTGAACCACATCCAGATCGAGTACAACAATATGCTGACTAAGTCCGGTGA
GT

**No57**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTTCATCAGGTCTGTTTACTACAACAAGTT
AGAATTCACGAGGTTCAGCAGCTCAGTGGGGAAGTTTGTCGGCTTCACTGAACGCGGAGTG
AAGAACGCTGAATACTGGAACAACGACCCTTCAAAAGTGAGTGCTATGAAGGCTCAGAAG
GAGGGTTACTGTCTGCACAACATCCAGATCGATTACACAGCTGCTCTGACTAAGTCCGGTG
AGT

**No58**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTTCATCAGGTCTTATTACTACAACAAGTT
AGAATTCACGAGGTTCAGCAGCTCAGTGGGGAAGTTTGTCGGCTTCACTGAGTACGGAGTG
AGGAACGCTGAATACTGGAACAACGACGCTTCACTTCTGAGTGCTATGAAGGCTCAGAAGG
AGGTTTACTGTCTGCACAACATCCAGATCTGGTACAACAATATGCTGACTAAGTCCGGTGA
GT

**No60**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTACATCCAGTCGTATTACTACAACAAGT
TAGAATACACGAGGTTCAGCAGCTCAGTGGGGAAGTTTGTCGGCTTCACTGAGTACGGAGT
GAAGAACGCTGAATACTGGAACAACGACGCTTCACTTCTGAGTGCTATGAAGGCTCAGAAG
GAGGCCGTCTGTCTGAACCACGTCCCGGTCTATTACAACAATATGCTGACTAAGTCCGGTG
AGT

**No61**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTTCATCGACTCGTATTACTTCAACAAGTT
AGAATACACGAGGTTCAGCAGCTCAGTGGGGAAGTATGTCGGCTTCACTGAACGCGGAGTG
AAGATCGCTGCTGACTGGAACAACAACCCTTCACTTCTGAGTGGAGAGAAGGCTCAGAAGG
AGGTTTACTGTCTGCACAACATCCAGATCTGGTACAACAATATTCTGACTAAGTCCGGTGA
GT

**No62**

GTCTTTAACTCCACGGAGCTGAAGGACATCGAGTACATCAGGTCTTATTACTACAACAAGT
TAGAATACACGAGGTTCAGCAGCTCAGTGGGGAAGTATGTCGGCTTCACTGAGTACGGAGT
GAGGATCGCTGCAGCCTGGAACAACAACCCTTCAATTCTGAGTAGAGCGAAGGCTCAGAA
GGAGGCTTACTGTCTGCACAACATCCAGATCGATTACAACAATATGCTGACTAAGTCCGGT
GAGT

**SCX03**

GAGTTCATCAGGTCTTATTACTTCAACAAGAAAGAAGACACGAGGTTCAGCAGCTCAGTGG
GGAAGTTTGTCGGCTTCACTGAACAAGGAGTGAGGAACGCTGCAGCCTGGAACAACAACC
CTTCAATTCTGAGTAATATGAAGGCTCAGAAGGAGGTTTACTGTCTGAACCACGTTCAGAT
CGATTACAACAATATTCTGACTAAGTCCG

**So11**

GAGTACATCAGGTCTTCTTACTTCAACAAGAAAGAAGACACGAGGTTCAGCAGCTCAGTGG
GGAAGTTTGTCGGCTTCACTGAACAAGGAGTGAAGTTCGCTGCAGCCTGGAACAACAACCC
TTCAATTCTGAGTAGAGCGAAGGCTCAGAAGGAGGTTTACTGTCTGAACCACGTCCCGGTC
TATTACAACAATATTCTGACTAAGTCCG

**Supplementary Information – Chapter 2**

*SUPPLEMENTARY METHODS - Breeding.* In August 2007, a total of six independent stickleback families (F1) were breed *in vitro* from wild stickleback caught at the Großer Plöner See (GPS; 54°9'21.16'N, 10°25'50.14'E, Germany). Juveniles were reared on a diet of *Artemia* (sp.) and copepods for the first six week before the diet was supplemented with frozen Chironomid larvae.

*Antigen preparation.* The antigen (AG) homogenate was composed in equal parts of *Diplostomum pseudospathaceum* and *Camallanus lacustris*. First, 8.5 ml *D. pseudospathaceum* and 3.4 ml *C. lacustris* were centrifuged in a cell strainer at 1000 rotations for 5 minutes and washed with 500 and 1600 µL of water respectively. Each homogenate was sonified on ice twice (at 30% duty cycle, output level 5) for 4 minutes, spun down at 4500 rotations and 4°C for 20 minutes. The antigen (AG) was prepared from 200 µL filtered parasite homogenate and 3.8 ml phosphate buffered saline (PBS) solution. The final AG solution was then synthesised from equal parts (1µg) of both parasites mixed with Freund's complete adjuvant to yield 4 µl per fish.

*Antigen injection.* In the first week of February (06/07.02.2008; ~26 weeks after hatching), about 40 individuals from each family were injected with 4 µL AG or PBS respectively (Figure 2). After a week, average mortality per family upon this first injection was 2.8 (±1.9SE) for AG-injected and 2.2 (±1.1SE) for PBS-injected fish (Welch T-test: *d.f.*=8.1, t=0.31, p=0.765). Five weeks post injection, the fish were first brought into artificial autumn (+12°C; 2 weeks) and then winter conditions (+6°C; 7 weeks; Fig 1A). Hence, the first injection mimics a first contact with parasites before they enter a relatively parasite free time during winter as would be the case under natural conditions. Hereafter, the fish were returned to artificial summer condition (+18°C) after a brief spring period (+12°C; 2 weeks). At this stage the fish received a second injection similar to that of the first one with AG or PBS respectively. Only a single mortality event was recorded following the second AG-injection.

*Laboratory-based experiments.* In order to establish the efficacy of the vaccination we first ran an experimental infection with one of the parasites used in the vaccination. A total of eight randomly chosen AG- and PBS-injected individuals from each family were exposed to a standardised amount of *D. pseudospathaceum* cercariae. The cercariae of *D. pseudospathaceum* infects their host actively and migrates to the eye lens within 24hrs past exposure(Rauch et al. 2006). Additionally, eight AG-injected (11 for one family) and PBS-injected (5 for two families) individuals served as uninfected control. All fish were kept under the same laboratory conditions for another 3 days and then each eye lens was screened and the fish measured and weighed. The spleen was removed, weighed and used to calculate the splenosomatic index: SSI=(spleen weight/body weight)*100(Kalbe et al. 2009). Total cell numbers in isolates of the head kidney, the major haemopoietic and lymphoid organ in fishes, were analysed with a flow cytometer (FACSCalibur, Becton and Dickinson, USA) to determine granulocyte and lymphocyte counts from all fish in the laboratory experiment following previously published protocols(Scharsack et al. 2007).

*Enclosure Experiments.* After the second injection all 192 fish (96 PBS/AG-injected fish from 6 families) were spin-clipped, weighed (initial weight), measured (initial standard length) and sexed. As to allow re-identification and parenthood assignment, DNA was extracted from the spine using DNeasy 96 Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. All fish were then genotyped for twelve microsatellites combined in three different multiplexed PCRs(Kalbe et al. 2009).

We released a total of 16 fish from the same family in one parasite-exposed and –unexposed enclosure each in equal sex ratio. Of the 16, eight fish had been injected with the AG, while eight with the PBS solution (Fig 1A). The lake enclosures (3 x 3m, height of 1m, 0.4-0.6m water depth) are made of stainless steel with 0.5cm mesh size and embedded in the lake floor of the Groβe Plöner See(Eizaguirre et al. 2009*b*; Kalbe et al. 2009; Lenz et al. 2009*b*). The parasite-free outside enclosures are concrete tanks of 4 x 4.85m, depth of up to 2.1m are located to the Max Planck Institute for

Evolutionary biology in Plön, Germany (Eizaguirre et al. 2012*b*). Those enclosures are fed by water from the lake Schöhsee The water was filtered with a 20 nm. Both environments experienced similar abiotic conditions (temperature difference ~1°C during the course of the experiment) with naturally-replenished food (lake: zooplankton/benthic prey; outside enclosures: chironomid larvae). The mesh size of the lake enclosures (0.5cm) allowed for the free passage of all intermediate hosts or actively infecting parasites. All enclosures allowed for natural mating dynamics, including male territoriality/nest building and female choice.

On a weekly basis, eggs were recovered from all nests, and clutches sorted by developmental stage and incubated in aerated well water at 18°C until dark eye spots were visible and the neutral tube was developed(Kalbe et al. 2009). This allowed for sufficient DNA for extraction. A total of 24 eggs from each clutch were randomly taken for DNA was extraction using Invisorb Tissue HTS 96 kit/R (Stratec) and a Freedom evo robot (Tecan). Fish were genotyped for twelve microsatellite markers, combined in three multiplexed PCRs(Eizaguirre et al. 2009*b*; Kalbe et al. 2009). Parenthood was assigned using the software PAPA(Duchesne et al. 2002) and used to determine lifetime reproductive success(Kalbe et al. 2009). In total we genotyped 3959 eggs from 139 clutches (parasite-exposed enclosure mean number of clutches ± standard error: 10.2 ±1.3; parasite-free enclosures: 12.2 ±2.3; students t-test: *d.f.*=7.9, t=0.76, p=0.469).

In week 9 after release into the enclosures, we recovered all fish from the enclosures. Subsequently, they were weighed, measured and screened for all external and internal parasites blind of the origin of the fish. We found on average 8.6 (±0.2 standard error) different parasite species on fish from the parasite-exposed enclosures, but no parasite infection in the parasite free environment. This confirmed fish from the parasite-free environment were not infected by macroparasites. Both, initial and final weights and lengths were used to calculate initial and final body condition: $BC=(weight/length)^{b}*100$ with *b* set at 3.00(Frischknecht 1993). The SSI and individual parasite load ($I_{PI}$) was calculating following Kalbe *et al.* (2002) and Kalbe *et al.* (2009).

*Data analyses – Laboratory experiment.* Differences in infection with *D. pseudospathaceum* (square-root transformed) amongst all parasite-exposed fish were tested using a mixed effect model with injection treatment (AG, PBS) and Body condition as fixed factors and family as well as sex as random factors. We tested for differences in SSI and lymphocyte count between injection treatments, *D. pseudospathaceum* exposure (yes/no), and standard length using another two mixed effect model with family and sex as random factors (Supplementary Table 2). We used Tukey post-hoc tests for pairwise comparison of all interactions.

*Data analyses – Enclosure experiment.* To assess differences in lifetime reproductive success (square-root transformed), we used a mixed effect model with injection treatment (AG, PBS), selection environment (parasite-free outside enclosures, parasite-exposed lake enclosures) and individual body condition as explanatory variables and family as well as sex as random factors. Similarly, we assessed the impact of those variables on SSI. Among parasite-exposed fish, the number of *D. pseudosathaceum* and *C. lacustris* was compared using a mixed effect model with injection treatment and body condition as explanatory variables and family and sex as random effects. Parasite load (log-transformed) was analysed with the same model. Survival was analysed using a generalised with injection treatment and selection environment as explanatory and family and sex as random factors. Body condition was also analysed but with a /linear mixed effect model using injection treatment and selection environment as explanatory and family and sex as random factors (Supplementary Table 3). Models were backward selected using the *anova* function. When the final model retained an interactions between injection treatment and selection environment we ran two mixed effect models for each selection environment separately: For the parasite-free environment such model contained the respective response variable and injection treatment and body condition. For parasite-exposed lake enclosures, parasite load (residuals corrected for body condition) was added. Family and sex remained random factors in these models.

*Mathematical model*. The trade-off between costs and benefits of adaptive immunity under different strengths of parasite-mediated selection, can simply be described following:

$$\dot{H} = (cost\ of\ vaccination * fitness - cost\ of\ parasitism * benefits\ of\ vaccination)$$

where $\dot{H}$ stands for absolute fitness or the change in frequency of groups with differential costs and benefits of vaccination.

We assumed two groups, one with adaptive immunity, capable of mounting memory cell-mediated response under the presence of parasites (the AG-injected group), and one without adaptive immunity (PBS-injected). Their population dynamics is defined by two differential equations:

$$\dot{H}_{AG\ t+1} = H_{AG\ t} * \left(\left(v * r - \beta * \frac{v}{m}\right) - \left(\left(v * r - \beta * \frac{v}{m}\right) * H_{AG\ t} + (r - \beta) * H_{PBS\ t}\right)\right)$$

$$\dot{H}_{PBS\ t+1} = H_{PBS\ t} * \left((r - \beta) - \left(\left(v * r - \beta * \frac{v}{m}\right) * H_{AG\ t} + (r - \beta) * H_{PBS\ t}\right)\right)$$

Here, the frequency of individuals with an adaptive immunity $H_{AG}$ and without $H_{PBS}$ are determined by differences in fitness *r* arising from the cost of immunity $v$, the strength of selection by parasites $\beta$, and the benefits from immunity $\frac{v}{m}$, which emerge from the modulation of parasite-mediated costs $(\beta - \frac{v}{m})$. In this case, $m$ describes a vector that minimises the impact of $v$ to represent the difference in fitness between PBS- and AG-injected fish under parasite exposure. The term $\left(\left(v * r = \beta * \frac{v}{m}\right) * H_{AG|PBS\ t} + (r - \beta) * H_{PBS|AG\ t}\right)$ ensures that $H_{AG} + H_{PBS} = 1$. For the purpose of this model, we assume linear costs and benefits of $m$, $v$, and $\beta$.

*EXTENDED RESULTS*. Under laboratory conditions we found a higher lymphocyte count in AG-injected fish regardless of whether they were infected with *D. pseudospathecum* (F1,178=37.28,

$p<0.001$; Supplementary Table 2). Due to the role of T and B lymphocytes in parasite-specific immune responses and long lasting immunological memory, these results underscore that AG-injection triggered the adaptive arm of immunity.

For those fish released to parasite-exposed enclosures, the AG-injection did not result in a difference in number of *C. lacustris* (reported as mean± standard error, AG: 1.5 ±0.1, PBS-injected: 1.4 ±0.1; $F_{1,23}=0.02$, $p=0.881$; Supplementary Table 1), likely due to overall low numbers of this parasite in the lake that year. Overall, survival was not different between injection treatments ($F_{1,188}=0.04$, $p=0.834$), but markedly differed between selection environments ($F_{1,188}=27.25$, $p<0.001$), where mortality was highest in the parasite-exposed group (Supplementary Table 3). Body condition was also consistently higher in the parasite unexposed environment ($F_{1,137}=202.33$, $p<0.001$; Supplementary Table 3).

**Supplementary Table 1. Statistical results assessing the impact of injection treatment (AG/PBS), selection environment (lake/outside enclosures) and body condition on SSI and specific parasite infection load.** All models were back selected using the *anova* function. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| **Data** | **SSI (splenosomatic index)** | ***d.f.*** | **F-value** | **p-value** |
|---|---|---|---|---|
| Across selection environments | **Injection treatment** | 1,134 | 5.69 | **0.018** |
| | **Selection environment** | 1,144 | 28.42 | **<0.001** |
| | **Body condition** | 1,142 | 0.18 | 0.676 |
| | **Inj. treatment x sel. environment** | 1,135 | 5.34 | **0.022** |
| | ***Pairwise comparison (Tukey)*** | Estimate (±SE) | | p-value |
| | **AG/Parasite + vs. PBS/Parasite +** | <0.01 (0.01) | | 1 |
| | **AG/Parasite + vs. AG/Parasite -** | 0.33 (0.11) | | **0.012** |
| | **AG/Parasite + vs. PBS/Parasite -** | 0.61 (0.11) | | **<0.001** |
| | **PBS/Parasite + vs. AG/Parasite -** | 0.32 (0.11) | | **0.020** |
| | **PBS/Parasite + vs. PBS/Parasite -** | 0.61 (0.11) | | **<0.001** |
| | **AG/Parasite - vs. PBS/Parasite -** | 0.28 (0.08) | | **0.002** |
| Parasite-exposed enclosures | **Injection treatment** | 1,52 | 0.14 | 0.707 |
| | **Body condition** | 1,46 | 0.15 | 0.704 |
| | **Parasite load (corrected with BC)** | 1,46 | 2.06 | 0.158 |
| Parasite-free enclosures | **Injection treatment** | 1,76 | 18.06 | **<0.001** |
| | **Body condition** | 1,84 | 1.38 | 0.244 |
| | ***Diplostomum pseudosathaceum*** | ***d.f.*** | **F-value** | **p-value** |
| Parasite-exposed enclosures | **Injection treatment** | 1,44 | 4.82 | 0.034 |
| | **Body condition** | 1,54 | 2.38 | 0.128 |
| | **Inj. Treatment x body condition** | 1,44 | 5.45 | **0.024** |
| | ***Camallanus lacustris*** | ***d.f.*** | **F-value** | **p-value** |
| Parasite-exposed enclosures | **Injection treatment** | 1,23 | 0.02 | 0.883 |
| | **Body condition** | 1,23 | 1.34 | 0.259 |

**Supplementary Table 2. Statistical results comparing the effect of injection treatment (AG/PBS), infected with *D. pseudosathaceum* (infected/uninfected) and standard length on infection success and activity of the adaptive arm of the immune system using SSI and lymphocyte counts as proxies.** All models were back selected using the *anova* function. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| **Data** | ***Diplostomum pseudosathaceum*** | ***d.f.*** | **F-value** | **p-value** |
|---|---|---|---|---|
| **Infected** | **Injection treatment** | 1,87 | 4.08 | **0.047** |
| | **Body Condition** | 1,93 | 3.59 | 0.061 |
| | **SSI (splenosomatic index)** | ***d.f.*** | **F-value** | **p-value** |
| **infected and uninfected** | **Injection treatment** | 1,178 | 5.96 | **0.016** |
| | **Body Condition** | 1,166 | 32.44 | **<0.001** |
| | ***D. pseudosathaceum* infection** | 1,176 | 1.11 | 0.293 |
| | **Lymphocyte count** | ***d.f.*** | **F-value** | **p-value** |
| **infected and uninfected** | **Injection treatment** | 1,178 | 37.28 | **<0.001** |
| | **Body Condition** | 1,181 | 66.35 | **<0.001** |
| | ***D. pseudosathaceum* infection** | 1,177 | 1.79 | 0.409 |

**Supplementary Table 3. Statistical results assessing the impact of injection treatment (AG/PBS), selection environment (lake/outside enclosures) on survival, body condition, and overall parasite load.** All models were back selected using the *anova* function. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| | **Survival** | ***d.f.*** | **F-value** | **p-value** |
|---|---|---|---|---|
| Across selection environments | **Injection treatment** | 1,188 | 0.04 | 0.834 |
| | **Selection environment** | 1,188 | 27.25 | **<0.001** |
| | **Body condition** | ***d.f.*** | **F-value** | **p-value** |
| Across selection environments | **Injection treatment** | 1,136 | 1.49 | 0.223 |
| | **Selection environment** | 1,137 | 202.33 | **<0.001** |
| | **Parasite load ($I_{PI}$)** | ***d.f.*** | **F-value** | **p-value** |
| Parasite-exposed enclosures | **Injection treatment** | 1,45 | 0.02 | 0.881 |
| | **Body condition** | 1,51 | 1.49 | 0.227 |

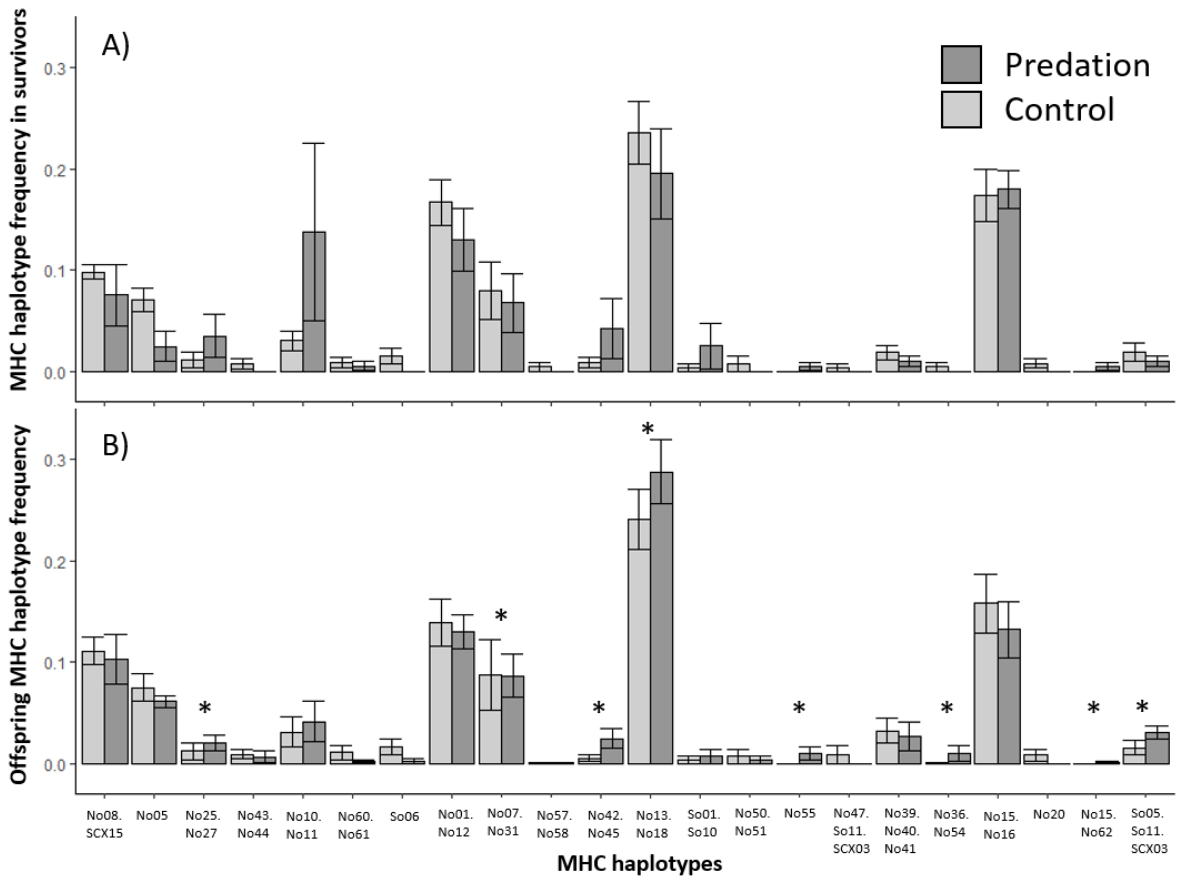
**Supplementary Information – Chapter 3**

**Supplementary Table S1. Summary table comparing (A) individual number of MHC alleles between years, treatments and among enclosures and (B) MHC haplotype pools between treatments within each year.**

| **A)** | **Number of MHC alleles** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **individual MHC diversity** | | | **Statistical summary** | | |
| | | | **median** | **Lowest** | **highest** | | | |
| Year | | 2011 | 4 (61.1%) | 2 (17.4%) | 5 (9.0%) | Wilcoxon rank test | | |
| | | 2012 | 4 (63.2%) | 1 (0.7%) | 5 (7.6%) | W=10 177, p=0.939 | | |
| Treat. | Predation | 2011 | 4 (59.7%) | 2 (16.7%) | 5 (11.1%) | Wilcoxon rank test | | |
| | Control | 2011 | 4 (62.5%) | 1 (1.4%) | 5 (6.9%) | W=2 408, p=0.600 | | |
| | Predation | 2012 | 4 (63.9%) | 2 (9.7%) | 5 (9.7%) | Wilcoxon rank test | | |
| | Control | 2012 | 4 (62.5%) | 2 (9.7%) | 5 (6.9%) | W=2 439, p=0.479 | | |
| Enclosure | | 2011 | | | | Kruskal-Wallis test | $X^2_5$=5.39 | p=0.369 |
| | | 2012 | | | | Kruskal-Wallis test | $X^2_5$=8.79 | p=0.118 |
| **B)** | **MHC variant constitution** | | | | | | | |
| Treatment | | 2011 | | | | ANOSIM | R= -0.010 | p=0.930 |
| | | 2012 | | | | ANOSIM | R= -0.007 | p=0.821 |

**Supplementary Table 2. Statistical summary tables reporting the effects of treatment, sex, body condition, MHC zygosity, parasite load and all two-way interactions on a) survival, b) individual parasite load, c) final body condition, d) individual lifetime reproductive success and e) nest ownership.** All models were backward selected using the *anova* function in R. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| **a) Survival** | ***d.f.*** | **F-value** | **p-value** |
|---|---|---|---|
| **Treatment** | 1,286 | 10.40 | **<0.001** |
| **Sex** | 1,286 | 10.69 | **0.001** |
| **Initial body condition** | 1,286 | 0.10 | 0.805 |
| **Zygosity** | 1,286 | 0.58 | 0.451 |
| | | | |
| **b) Individual Parasite Load ($I_{PI}$)** | | | |
| **Sex** | 1,169 | 1.18 | 0.279 |
| **Treatment** | 1,141 | 7.67 | **0.006** |
| **Initial body condition** | 1,171 | 0.18 | 0.676 |
| **Zygosity** | 1,169 | 0.87 | 0.352 |
| **Treatment * Initial body condition** | 1,171 | 5.88 | **0.016** |
| | | | |
| **c) Final Body Condition** | | | |
| **Sex** | 1,170 | 66.20 | **<0.001** |
| **Treatment** | 1,9 | 0.86 | 0.378 |
| **Parasite load corrected for initial body condition** | 1,153 | 2.63 | 0.107 |
| **Initial body condition** | 1,176 | 60.12 | **<0.001** |
| **Zygosity** | 1,169 | 4.46 | **0.036** |
| | | | |
| **d) Lifetime reproductive success (LRS)** | | | |
| **Sex** | 1,168 | 0.06 | 0.807 |
| **Treatment** | 1,5 | 2.93 | 0.152 |
| **Parasite load corrected for initial body condition** | 1,46 | 9.31 | **0.004** |
| **Initial body condition** | 1,116 | 7.31 | **0.008** |
| **Zygosity** | 1,173 | 0.44 | 0.508 |
| **Treatment * Parasite load corrected for initial body condition** | 1,58 | 3.23 | 0.078 |
| | | | |
| **e) Nest ownership** | | | |
| **Treatment** | 1,82 | 1.30 | 0.272 |
| **Initial body condition** | 1,82 | 4.50 | 0.072 |
| **Zygosity** | 1,82 | 0.43 | 0.515 |
| **Parasite load corrected for initial body condition** | 1,82 | 10.09 | **0.001** |




**Supplementary Figure 1. A) MHC haplotype frequency of survivors is not significantly different between treatments (control: light grey; predation: dark grey). B) Offspring MHC haplotype frequency is significantly different between treatment groups.** Asterisks denotes MHC haplotypes contributing significantly to this difference ($p<0.05$; Supplementary Table 3).

**Supplementary Table 3. Results of Similarity Percentage Analysis (SIMPER) between MHC haplotype pools in offspring from the control and predation treatments.** Average contribution to overall dissimilarity (average), standard deviation (sd); average abundance per group (Predation: $average_p$, Control: $average_c$); ordered cumulative contribution (Cum. sum), permutation p-value (p) are shown.

| MHC Haplotypes | average | sd | $average_P$ | $average_C$ | Cum sum | p | NCBI Accession # |
|---|---|---|---|---|---|---|---|
| **No18.No13** | 0.11 | 0.097 | 1.103 | 0.946 | 0.165 | <**0.010** | AF395711/ AY687846 |
| **No01.No12** | 0.088 | 0.091 | 0.528 | 0.545 | 0.296 | 0.98 | DQ016399/016400 |
| **No15.No16** | 0.086 | 0.089 | 0.484 | 0.59 | 0.425 | 1 | DQ016410/015617 |
| **No08.SCX15** | 0.073 | 0.079 | 0.451 | 0.433 | 0.533 | 0.436 | AY687842/ EU541449 |
| **No07.No31** | 0.072 | 0.09 | 0.401 | 0.36 | 0.641 | <**0.010** | DQ016421/016406 |
| **No05** | 0.052 | 0.069 | 0.243 | 0.293 | 0.718 | 1 | AY687829 |
| **No39.No40.No41** | 0.03 | 0.056 | 0.133 | 0.145 | 0.763 | 0.911 | AAY34959/XXX*/XXX* |
| **No10.No11** | 0.03 | 0.056 | 0.139 | 0.136 | 0.812 | 0.376 | AF395722/AY587843 |
| **So05.So11.SCX03** | 0.02 | 0.047 | 0.128 | 0.05 | 0.849 | <**0.010** | DQ016402 /016404/AJ230191 |
| **No25.No27** | 0.016 | 0.042 | 0.087 | 0.049 | 0.881 | <**0.010** | AY687855/DQ016402 |
| **No42.No45** | 0.016 | 0.042 | 0.108 | 0.021 | 0.915 | <**0.010** | FJ360536/360537 |
| **So06** | 0.01 | 0.035 | 0.004 | 0.08 | 0.93 | 1 | FJ360531 |
| **No60.No61** | 0.007 | 0.029 | 0.012 | 0.046 | 0.941 | 1 | XXX*/XXX* |
| **No43.No44** | 0.007 | 0.029 | 0.014 | 0.043 | 0.951 | 1 | FJ360532/360533 |
| **No50.No51** | 0.007 | 0.035 | 0.022 | 0.031 | 0.961 | 1 | XXX*/XXX* |
| **No55** | 0.006 | 0.026 | 0.046 | 0 | 0.97 | <**0.010** | XXX* |
| **No20** | 0.006 | 0.026 | 0 | 0.045 | 0.978 | 1 | XXX* |
| **No36.No54** | 0.005 | 0.035 | 0.042 | 0.001 | 0.986 | <**0.010** | DQ016411/XXX* |
| **No47.So11.SCX03** | 0.005 | 0.025 | 0 | 0.041 | 0.993 | 1 | AJ230191/XXX*/XXX* |
| **So01.So10** | 0.003 | 0.019 | 0.011 | 0.015 | 0.998 | 1 | FJ360535/FJ360534 |
| **No57.No58** | 0.001 | 0.009 | 0.002 | 0.003 | 0.999 | 0.931 | XXX*/XXX* |
| **No15.No62** | 0.001 | 0.008 | 0.005 | 0 | 1 | <**0.010** | DQ016410/XXX* |

*for sequence see appendix 1

**Supplementary Table 4**. **Results of Similarity Percentage Analysis (SIMPER) between MHC haplotypes of regular nest owners (>=4 nesting events) from the control and predation treatment.** Average contribution to overall dissimilarity (average), standard deviation (sd); average abundance per group (Predation: $average_p$; Control: $average_c$); ordered cumulative contribution (Cum. sum), and permutation p-value (p) are shown.

| | overall acquiring nesting opportunities often | | | | | | |
|---|---|---|---|---|---|---|---|
| **Haplotype** | average | sd | ratio | average(P) | average(C) | cumsum | p |
| **No13.No18** | 0.221 | 0.139 | 1.595 | 0.889 | 0.176 | 0.287 | **0.001** |
| **No08.SCX15** | 0.147 | 0.148 | 0.996 | 0.333 | 0.529 | 0.478 | **0.001** |
| **No01.No12** | 0.108 | 0.152 | 0.709 | 0.222 | 0.235 | 0.618 | 0.840 |
| **No05** | 0.095 | 0.135 | 0.705 | 0.111 | 0.294 | 0.741 | 0.238 |
| **No07.No31** | 0.056 | 0.112 | 0.497 | 0.111 | 0.118 | 0.813 | 0.828 |
| **So06** | 0.039 | 0.112 | 0.352 | 0.000 | 0.118 | 0.864 | 0.408 |
| **No15.No16** | 0.039 | 0.112 | 0.352 | 0.000 | 0.118 | 0.915 | 0.996 |
| **No10.No11** | 0.033 | 0.091 | 0.360 | 0.000 | 0.118 | 0.965 | 0.118 |
| **No39.No40.No41** | 0.016 | 0.066 | 0.247 | 0.000 | 0.059 | 1.000 | 0.389 |

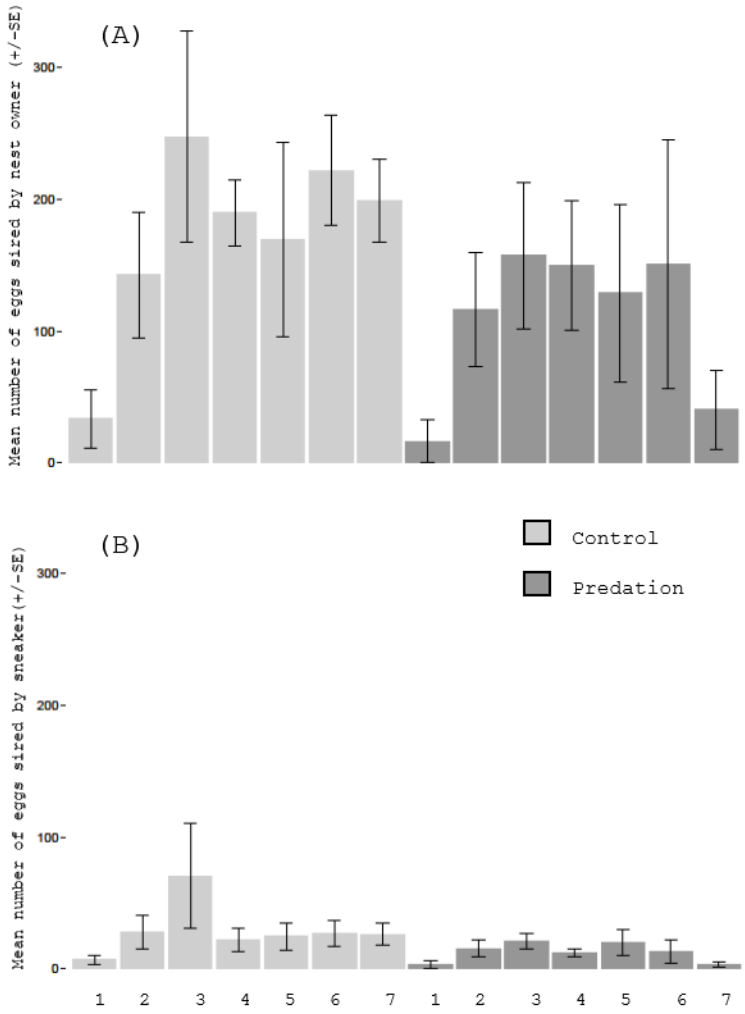
**Supplementary Table 5. Statistical summary table testing the effect of treatment, the presence and absence of MHC haplotype No08.SCX15 and the tested explanatory variables on a) survival, b) parasite load, c) final body condition, d) lifetime reproductive success and e) nest ownership in 2011**. All models were backward selected using the *anova* function in R. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| **a) Survival** | ***d.f.*** | **F-value** | **p-value** |
|---|---|---|---|
| **Sex** | 1,285 | 7.32 | **0.001** |
| **Treatment** | 1,285 | 13.55 | **<0.001** |
| **Initial body condition** | 1,285 | 0.09 | 0.798 |
| **Haplotype No08.SCX15** | 1,285 | 0.24 | 0.632 |
| **Zygosity** | 1,285 | 0.58 | 0.417 |
| | | | |
| **b) Individual Parasite Load ($I_{PI}$)** | | | |
| **Sex** | 1,85 | 0.07 | 0.791 |
| **Treatment** | 1,2 | 0.96 | 0.418 |
| **Initial body condition** | 1,87 | 0.29 | 0.591 |
| **Haplotype No08.SCX15** | 1,86 | 7.89 | **0.006** |
| **Zygosity** | 1,86 | 0.07 | 0.788 |
| | | | |
| **c) Final Body Condition** | | | |
| **Sex** | 1,84 | 58.89 | **<0.001** |
| **Treatment** | 1,2 | 0.20 | 0.695 |
| **Initial body condition** | 1,88 | 31.34 | **<0.001** |
| **Haplotype No08.SCX15** | 1,88 | 6.64 | **0.012** |
| **Zygosity** | 1,85 | 0.10 | 0.756 |
| **Parasite load corrected for initial body condition** | 1,86 | 0.01 | 0.956 |
| | | | |
| **d) Lifetime reproductive success (LRS)** | | | |
| **Sex** | 1,84 | 1.87 | 0.175 |
| **Treatment** | 1,3 | 3.37 | 0.157 |
| **Initial body condition** | 1,87 | 0.02 | 0.881 |
| **Haplotype No08.SCX15** | 1,86 | 6.46 | **0.013** |
| **Zygosity** | 1,85 | 0.07 | 0.795 |
| **Parasite load corrected for initial body condition** | 1,85 | 0.11 | 0.743 |
| **Treatment * Parasite load corrected for initial body condition** | 1,87 | 5.09 | **0.027** |
| | | | |
| **e) Nest ownership** | | | |
| **Treatment** | 1,82 | 1.30 | 0.203 |
| **Initial body condition** | 1,82 | 4.03 | 0.093 |
| **Haplotype No08.SCX15** | 1,82 | 9.26 | **0.002** |
| **Zygosity** | 1,82 | 0.34 | 0.240 |
| **Parasite load corrected for initial body condition** | 1,82 | 10.02 | **0.003** |

**Supplementary Information – Chapter 4**

**Supplementary Table S1. Summary table comparing (A) number of MHC alleles between years, sex, treatment (Treat.) and across enclosures and (B) MHC constitution between treatments within each year;**

| **A)** | **Number of MHC alleles** | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | **individual MHC diversity** | | | **Statistical summary** | |
| | | | **median** | **Lowest** | **highest** | | |
| Year | | 2011 | 4 (61.1%) | 2 (17.4%) | 5 (9.0%) | Wilcoxon rank test | |
| | | 2012 | 4 (63.2%) | 1 (0.7%) | 5 (8.3%) | W=10 054, p=0.779 | |
| Sex | Male | 2011 | 4 (69.4%) | 2 (13.9%) | 5 (8.3%) | Wilcoxon rank test | |
| | Female | 2011 | 4 (52.8%) | 1 (1.4%) | 5 (9.7%) | W=2 246, p=0.199 | |
| | Male | 2012 | 4 (59.7%) | 2 (12.5%) | 5 (6.9%) | Wilcoxon rank test | |
| | Female | 2012 | 4 (66.7%) | 2 (6.9%) | 5 (9.7%) | W=2 874, p=0.190 | |
| Treatment | Predation | 2011 | 4 (59.7%) | 2 (16.7%) | 5 (11.1%) | Wilcoxon rank test | |
| | Control | 2011 | 4 (62.5%) | 1 (1.4%) | 5 (6.9%) | W=2 408, p=0.600 | |
| | Predation | 2012 | 4 (63.9%) | 2 (9.7%) | 5 (9.7%) | Wilcoxon rank test | |
| | Control | 2012 | 4 (62.5%) | 2 (9.7%) | 5 (6.9%) | W=2 439, p=0.479 | |
| Enclosure | | 2011 | | | | Kruskal-Wallis test | $X^2$=5.39 p=0.369 |
| | | 2012 | | | | Kruskal-Wallis test | $X^2$=8.79 p=0.118 |
| **B)** | **MHC variant constitution** | | | | | | |
| Treatment | | 2011 | | | | ANOSIM | R= -0.010 p=0.930 |
| | | 2012 | | | | ANOSIM | R= -0.007 p=0.821 |




**Supplementary Figure 1 (A) Average number of eggs analysed for parenthood and (B) average number of eggs allocated to sneaker males per week.**

**Supplementary Table S2. Summary statistics evaluating the effects of sex, treatment and initial body condition on survival using a generalised linear model.** Models were backward selected using the *anova* function. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| explanatory variable | *d.f.* | F-value | p-value |
|---|---|---|---|
| **Treatment** | 1, 285 | 10.30 | **<0.001** |
| **Sex** | 1, 285 | 10.77 | **0.001** |

**Supplementary Table S3. Results from a SIMPER comparing parasite species abundances between treatment and control in 2011 (left) and 2012 (right).** Bolded species cumulatively account for more than 90% of the difference between years.

| species | Mean abundance | | % | species | Mean abundance | | % |
|---|---|---|---|---|---|---|---|
| | Control | Predation | | | Control | Predation | |
| ***Apiosoma* sp.** | **165.4** | **151.5** | **31.4** | ***Apiosoma* sp.** | **205.5** | **237.5** | **49.4** |
| ***Trichodina* sp.** | **114.8** | **92.6** | **20.9** | ***Trichodina* sp.** | **135.8** | **69.7** | **27.7** |
| ***Diplostomum pseudospathaceum*** | **48.1** | **48.9** | **9.6** | ***Echinochasmus* sp.** | **19.8** | **13.5** | **7.6** |
| ***Valipora campylancristrota*** | **26.5** | **24.8** | **8.2** | ***Diplostomum pseudospathaceum*** | **36.2** | **22.6** | **7.4** |
| ***Gyrodactylus* sp.** | **8.4** | **26.3** | **8** | ***Gyrodactylus* sp.** | **11.9** | **8.0** | **5.1** |
| ***Cyathocothyle prussica*** | **17.7** | **14.0** | **5** | Argulus | 2.7 | 2.5 | 1 |
| ***Echinochasmus* sp.** | **12.8** | **11.4** | **4** | ***Valipora campylancristrota*** | **0.6** | **0.9** | **<0.5** |
| *Camallanus lacustris* | 12.7 | 11.1 | 3.6 | *Diphyllobothrium* sp. | 0.3 | 0.6 | <0.5 |
| Glochidia | 0.4 | 11.9 | 3 | *Contracaecum* sp. | 0.4 | 0.6 | <0.5 |
| *Phyllodistomum folium* | 7.1 | 5.5 | 2.7 | *Apatemon cobitidis* | 0.6 | 0.1 | <0.5 |
| **Followed by:** *Proteocephalus filicollis, Argulus foliaceus, Apatemon cobitidis, Contracaecum* sp., *Anguillicola crassus, Paradilepsis scolecina, Tylodelphis clavata, Crepidostomum* sp., *Raphidascaris acus, Ergasilus* sp. | | | | **Followed by:** *Camallanus lacustris, Anguillicola crassus, Phyllodistomum folium, Tylodelphis clavata, Raphidascaris acus, Paradilepsis scolecina* | | | |
| **Absent:** *Diphyllobothrium* sp. | | | | **Absent:** Glochidia, ***Cyathocothyle prussica,*** *Ergasilus* sp., *Proteocephalus filicollis, Crepidostomum* sp. | | | |

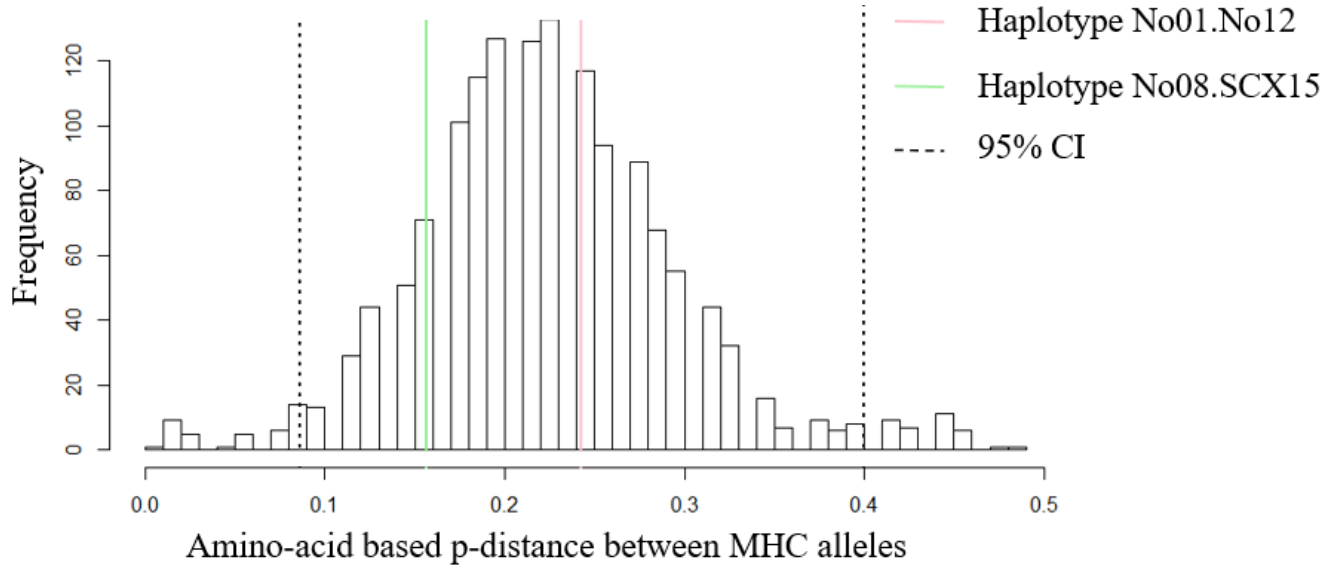
**Supplementary Table S4. Summary statistics documenting the effects of treatment, sex, MHC diversity/MHC haplotypes, initial body condition and all two-way interaction with treatment on individual parasite load.** Models were backward selected using the *anova* function. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| data | explanatory variable | *d.f.* | F-value | p-value |
|---|---|---|---|---|
| **both years** | **Treatment** | 1, 140 | 6.58 | **0.011** |
| | **Sex** | 1, 166 | 1.30 | 0.257 |
| | **MHC diversity** | 4, 165 | 0.89 | 0.473 |
| | **Initial Body Condition** | 1, 167 | 0.32 | 0.570 |
| | **Treatment * Initial Body Condition** | 1, 167 | 4.73 | **0.031** |
| **2011** | **Treatment** | 1, 2 | 0.83 | 0.454 |
| | **Sex** | 1, 86 | 0.01 | 0.932 |
| | **Haplotype No01.No12** | 1, 86 | 5.03 | **0.028** |
| | **Initial Body Condition** | 1, 88 | 1.46 | 0.230 |
| | **Treatment** | 1, 2 | 0.96 | 0.418 |
| | **Sex** | 1, 86 | 0.06 | 0.807 |
| | **Haplotype No08.SCX15** | 1, 87 | 7.99 | **0.006** |
| | **Initial Body Condition** | 1, 88 | 0.46 | 0.497 |

**Supplementary Table S5 A) Summary from SIMPER comparing MHC variants constitution across treatment within each year in order to find most frequent (>10%) MHC haplotypes and B) summary statistics measuring the effect of these haplotypes on resistance towards common parasites species.** Table presents only significant effects of haplotypes towards resistance against specific parasites. False discovery rate corrections ('fdr') was applied to account for at least 4 comparisons. d.f. denotes degrees of freedom.

| A) | MHC – 2011 | | | MHC – 2012 | | |
|---|---|---|---|---|---|---|
| **Haplotype** | **Frequency** | | **%** | **Frequency** | | **%** |
| | **Cont.** | **Pred.** | | **Cont.** | **Pred.** | |
| **No13.No18** | 34 | 32 | 19.3 | 32 | 34 | 17.8 |
| **No01.No12** | 22 | 18 | 17.3 | 20 | 17 | 13.7 |
| **No15.No16** | 23 | 27 | 15.8 | 19 | 15 | 12.7 |
| **No08.SCX15** | 13 | 12 | 10.9 | 12 | 15 | 10.7 |

| B) | **Parasite Species - *Valipora campylancristrota*** | | | | |
|---|---|---|---|---|---|
| **data** | **explanatory variable** | ***d.f.*** | **f-value** | **p-value** | **fdr' p-value** |
| | **Haplotype No08.SCX15** | 1,89 | 4.34 | **0.040** | **0.2** |
| 2011 | **Treatment** | 1,3 | 0.75 | 0.447 | |
| | **Interaction** | 1, 89 | 1.01 | 0.318 | |
| | **Parasite Species - *Gyrodactylus* sp.** | | | | |
| **data** | **explanatory variable** | ***d.f.*** | **f-value** | **p-value** | **fdr' p-value** |
| | **Haplotype No01.No12** | 1, 89 | 4.23 | **0.043** | 0.21 |
| 2011 | **Treatment** | 1, 4 | 2.42 | 0.195 | |
| | **Interaction** | 1, 89 | 8.12 | 0.005 | |
| | **Parasite Species - *Echinochasmus* sp.** | | | | |
| **data** | **explanatory variable** | ***d.f.*** | **f-value** | **p-value** | **fdr' p-value** |
| | **Haplotype No13.No18** | 1, 85 | 5.92 | **0.017** | 0.085 |
| 2012 | **Treatment** | 1, 5 | 0.14 | 0.723 | |
| | **Interaction** | 1, 85 | 1.95 | 0.166 | |




**Supplementary Figure 2. Individual amino-acid based p-distance estimates between alleles of the Haplotype No01.No12 (pink) and No08.SCX15 (green) in contrast to overall distribution of amino-acid based p-distance range of all MHC alleles in our sample population.**

**Supplementary Table S6. Anova results documenting the effects of treatment, sex, MHC diversity/MHC haplotypes, initial body condition, and parasite load ($I_{PI}$) and all two-way interaction with treatment on individual life-time reproductive success.** Models were backward selected using the *anova* function. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| data | explanatory variable | *d.f.* | F-value | p-value |
|---|---|---|---|---|
| both years | **Treatment** | 1, 19 | 5.99 | **0.025** |
| | **MHC diversity** | 4, 167 | 0.96 | 0.434 |
| | **Initial Body Condition** | 1, 172 | 0.57 | 0.453 |
| | **Sex** | 1, 166 | 0.06 | 0.801 |
| | **$I_{PI}$** | 1, 105 | 4.33 | **0.040** |
| | **Treatment * $I_{PI}$** | 1, 89 | 4.55 | **0.036** |
| 2011 | **Treatment** | 1, 87 | 4.30 | **0.041** |
| | **Haplotype No01.No12** | 1, 83 | 4.35 | **0.040** |
| | **Initial Body Condition** | 1, 87 | 0.65 | 0.424 |
| | **Sex** | 1, 84 | 1.65 | 0.202 |
| | **$I_{PI}$** | 1, 86 | 0.41 | 0.526 |
| | **Treatment * Initial Body Condition** | 1, 87 | 4.51 | **0.037** |
| | **Treatment * $I_{PI}$** | 1, 86 | 4.90 | **0.030** |
| | **Treatment** | 1, 87 | 3.20 | 0.077 |
| | **Haplotype No08.SCX15** | 1, 85 | 6.58 | **0.012** |
| | **Initial Body Condition** | 1, 87 | 0.07 | 0.797 |
| | **Sex** | 1, 84 | 2.43 | 0.122 |
| | **$I_{PI}$** | 1, 87 | 0.10 | 0.758 |
| | **Treatment * Initial Body Condition** | 1, 87 | 3.36 | 0.070 |
| | **Treatment * $I_{PI}$** | 1, 86 | 3.89 | 0.052 |

**Supplementary Table 7. Summary statistics documenting the effect of treatment, lifetime reproductive success (LRS), initial body condition, parasite load ($I_{PI}$), individual MHC diversity and all two-way interactions with predation treatment on individual number of eggs sneak fertilised.** Models were backward selected using the *anova* function. Significant results are highlighted in bold. d.f. denotes degrees of freedom.

| data | Explanatory Variables | *d.f.* | F-value | p-value |
|---|---|---|---|---|
| both years | **Treatment** | 1, 26 | 7.46 | **0.011** |
| | **LRS** | 1, 68 | 36.42 | **<0.001** |
| | **Initial Body Condition** | 1, 67 | 1.83 | 0.181 |
| | **$I_{PI}$** | 1, 66 | 1.01 | 0.320 |
| | **MHC diversity** | 3, 65 | 0.34 | 0.797 |
| | **LRS * Treatment** | 1, 67 | 10.22 | **0.002** |
| 2011 | **Treatment** | 1, 39 | 6.07 | **0.018** |
| | **LRS corrected for Hap. No08.SCX15** | 1, 40 | 28.70 | **<0.001** |
| | **Initial Body Condition** | 1, 39 | 0.25 | 0.623 |
| | **$I_{PI}$** | 1, 40 | 2.46 | 0.124 |
| | **Haplotype No08.SCX15** | 1, 40 | 4.93 | **0.032** |
| | **Treatment * Initial Body Condition** | 1, 40 | 6.67 | **0.014** |
| | **Treatment** | 1, 39 | 3.82 | 0.058 |
| | **LRS corrected for Hap. No01.No12** | 1, 40 | 20.66 | **<0.001** |
| | **Initial Body Condition** | 1, 40 | 0.39 | 0.534 |
| | **$I_{PI}$** | 1, 40 | 3.33 | 0.076 |
| | **Haplotype No01.No12** | 1, 40 | 17.14 | **<0.001** |
| | **Treatment * Initial Body Condition** | 1, 40 | 4.55 | **0.039** |

**Supplementary Table 8. Mann-Whitney U and Kolmogorov Smirnov tests result comparing observed versus random mate choice with regards to (A) MHC variant sharing value and (B) relatedness from all reproductively active individuals across all breeding seasons.** Values represent number of times the p-value was below the 0.05 significance threshold. Bolded values conform to the 95% confidence interval rule.

| Year | Enclosures | entire breeding period | | 1st breeding period | | 2nd breeding period | |
|---|---|---|---|---|---|---|---|
| | | wilcox<0.05 | ks<0.05 | wilcox<0.05 | ks<0.05 | wilcox<0.05 | ks<0.05 |
| (A) Mate choices with regards to MHC variant sharing value | | | | | | | |
| 2011 | P1 | 86 | 9 | **1000** | **1000** | 70 | 73 |
| | C2 | 450 | 71 | **961** | 712 | 918 | 831 |
| | P3 | **991** | **953** | **1000** | **1000** | 771 | **1000** |
| | C4 | 933 | **919** | **953** | **985** | 56 | 252 |
| | P5 | 67 | 28 | 116 | 96 | 93 | 26 |
| | C6 | 274 | 389 | **1000** | **1000** | 683 | 624 |
| 2012 | C1 | 54 | 21 | **1000** | **1000** | 256 | 237 |
| | P2 | **955** | **972** | **995** | **1000** | 64 | 695 |
| | C3 | 133 | 79 | **999** | **1000** | 44 | 9 |
| | P4 | 71 | 11 | **998** | **1000** | 918 | 864 |
| | C5 | 720 | 617 | **952** | **1000** | **1000** | **1000** |
| | P6 | 627 | 676 | **986** | **987** | **998** | **994** |
| (B) Mate choices with regards to background relatedness | | | | | | | |
| 2011 | P1 | 59 | 790 | 735 | **1000** | 699 | 891 |
| | C2 | 126 | 49 | 345 | 914 | 337 | **985** |
| | P3 | 116 | **1000** | 525 | **1000** | **993** | **1000** |
| | C4 | 491 | 934 | 697 | **996** | 55 | 945 |
| | P5 | 894 | **1000** | **989** | **1000** | **966** | **996** |
| | C6 | 746 | 906 | **1000** | **1000** | 335 | **952** |
| 2012 | C1 | 184 | **984** | **1000** | **1000** | 323 | 890 |
| | P2 | 159 | **996** | **1000** | **1000** | 847 | **1000** |
| | C3 | 79 | 110 | 209 | **1000** | 138 | 385 |
| | P4 | 114 | 480 | 891 | **994** | 750 | **985** |
| | C5 | **988** | **995** | 84 | 816 | **992** | **1000** |
| | P6 | 46 | 644 | 687 | **1000** | 86 | 609 |

**Supplementary Table 9. Results from a generalised linear model estimating the effects of presence/absence of MHC haplotype No08.SCX15 on mate choice between treatments.** Significant results are highlighted in bold. d.f. denotes degrees of freedom.

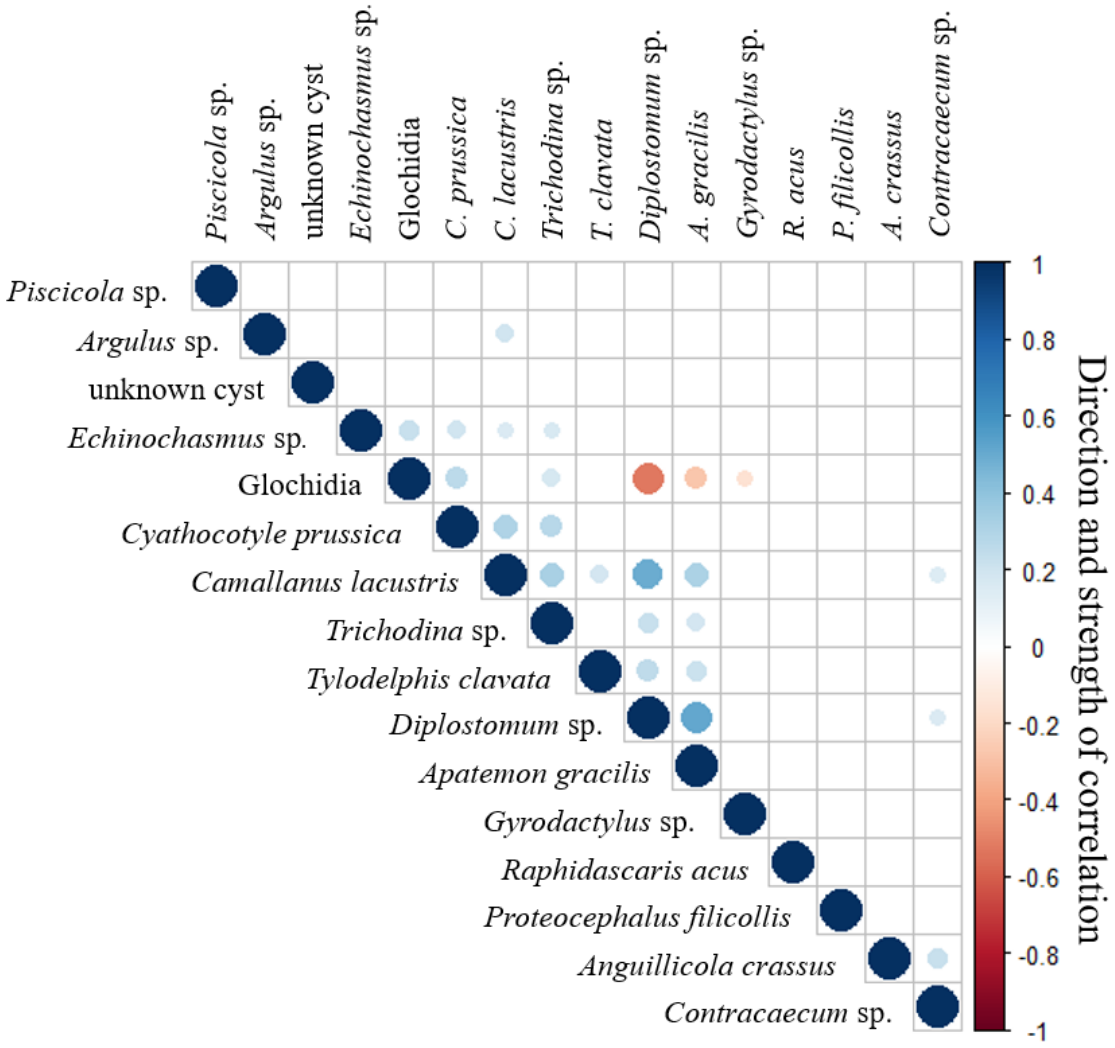
| data | Explanatory Variable | *d.f.* | F-value | p-value |
|---|---|---|---|---|
| Entire breeding season | **Haplotype No08.SCX15** | 1,689 | 10.49 | **0.001** |
| | **treatment** | 1,689 | 0.04 | 0.838 |
| | **Haplotype No08.SCX15 * Treatment** | 1,689 | 13.36 | **<0.001** |
| 1st breeding period | **Haplotype No08.SCX15** | 1,621 | 2.88 | 0.090 |
| | **Treatment** | 1,621 | 0.08 | 0.777 |
| | **Haplotype No08.SCX15 * Treatment** | 1,621 | 6.23 | **0.013** |
| 2nd breeding period | **Haplotype No08.SCX15** | 1,528 | 13.42 | **<0.001** |
| | **Treatment** | 1,528 | 2.14 | 0.144 |
| | **Haplotype No08.SCX15 * Treatment** | 1,528 | 22.53 | **<0.001** |

**Supplementary Information – Chapter 5**

**Supplementary Table 2. Results from a similarity percentage analysis (SIMPER) identify those parasites associated with differences between sequential seasons.** Parasite species with significant differences and statistically relevant sample size are highlighted in bold. Average contribution to overall dissimilarity (average), standard deviation (SD); average abundance per group; ordered cumulative contribution (cumsum), and permutation p-value are shown. Glochidia and *Anguillicola crassus* were not present in summer and autumn.

| | **Average (±SD)** | **Average Spring** | **Average Summer** | **cumsum** | **p-value** |
|---|---|---|---|---|---|
| **Glochidia** | **0.18 (0.06)** | **2.36** | **0.00** | **0.29** | **0.001** |
| ***Diplostomum* sp.** | **0.17 (0.06)** | **0.91** | **3.09** | **0.57** | **0.001** |
| *Camallanus lacustris* | 0.06 (0.05) | 0.57 | 1.14 | 0.67 | 1.000 |
| *Cyathocotyle prussica* | 0.06 (0.04) | 0.87 | 0.79 | 0.76 | 1.000 |
| ***Apatemon gracilis*** | **0.04 (0.04)** | **0.04** | **0.54** | **0.83** | **0.001** |
| *Trichodina* sp | 0.04 (0.03) | 0.71 | 0.79 | 0.88 | 1.000 |
| *Echinochasmus* sp. | 0.03 (0.04) | 0.31 | 0.20 | 0.93 | 0.093 |
| *Tylodelphis clavata* | 0.01 (0.03) | 0.03 | 0.16 | 0.95 | 0.117 |
| *Proteocephalus filicollis* | 0.01 (0.02) | 0.09 | 0.05 | 0.97 | 0.060 |
| *Argulus* sp. | 0.01 (0.02) | 0.05 | 0.06 | 0.98 | 0.038 |
| ***Contracaecum* sp.** | **0.01 (0.01)** | **0.01** | **0.05** | **0.99** | **0.011** |
| *Gyrodactylus* sp | 0.00 (0.01) | 0.01 | 0.04 | 0.99 | 1.000 |
| *Raphidascaris acus* | 0.00 (0.01) | 0.00 | 0.03 | 0.99 | 0.588 |
| unknown cyst | 0.00 (0.01) | 0.02 | 0.00 | 1.00 | 0.364 |
| *Anguillicola crassus* | 0.00 (0.01) | 0.02 | 0.00 | 1.00 | 0.365 |
| *Piscicola* sp. | 0.00 (0.00) | 0.00 | 0.00 | 1.00 | 0.531 |
| | **Average (±SD)** | **Average Summer** | **Average Autumn** | **cumsum** | **p-value** |
| ***Diplostomum* sp.** | **0.23 (0.10** | **3.09** | **1.18** | **0.35** | **0.001** |
| ***Camallanus lacustris*** | **0.13 (0.07)** | **1.14** | **0.02** | **0.55** | **0.001** |
| *Cyathocotyle prussica* | 0.08 (0.07) | 0.79 | 0.18 | 0.68 | 0.001 |
| *Trichodina* sp | 0.07 (0.05) | 0.79 | 0.23 | 0.79 | 0.001 |
| ***Apatemon gracilis*** | **0.06 (0.06)** | **0.54** | **0.02** | **0.88** | **0.001** |
| *Echinochasmus* sp. | 0.02 (0.04) | 0.20 | 0.01 | 0.91 | 1.000 |
| ***Tylodelphis clavata*** | **0.02 (0.04)** | **0.16** | **0.03** | **0.94** | **0.001** |
| ***Gyrodactylus* sp** | **0.02 (0.03)** | **0.04** | **0.12** | **0.97** | **0.002** |
| *Proteocephalus filicollis* | 0.01 (0.02) | 0.05 | 0.00 | 0.97 | 0.997 |

| | | | | | |
|---|---|---|---|---|---|
| ***Contracaecum* sp.** | **0.01 (0.02)** | **0.05** | **0.00** | **0.98** | **0.001** |
| *Argulus* sp. | 0.01 (0.02) | 0.06 | 0.00 | 0.99 | 0.593 |
| *Raphidascaris acus* | 0.01 (0.02) | 0.03 | 0.01 | 1.00 | 0.001 |
| unknown cyst | 0.00 (0.00) | 0.00 | 0.00 | 1.00 | 0.988 |
| *Piscicola* sp. | 0.00 (0.00) | 0.00 | 0.00 | 1.00 | 0.584 |




**Supplementary Figure 1. Correlation matrix for all parasite species after Bonferroni correction.**

**Supplementary Table 3. Summary for test results of PERMANOVA analyses to test the infection (high vs. low) of all MHC haplotypes (as matrix) with three parasite species.** Significant results are highlighted in bold; d.f. denotes degrees of freedom.

| (A) | Parasite - *Diplostomum* sp. | | | |
|---|---|---|---|---|
| **Season** | **PERMANOVA** | | | |
| | explanatory variable | *d.f.* | F-value | p-value |
| Spring | **Infection (low vs. high)** | **1,161** | **3.181** | **0.006** |
| Summer | **Infection (low vs. high)** | **1,162** | **4.230** | **<0.001** |
| Autumn | **Infection (low vs. high)** | **1, 126** | **3.118** | **0.009** |
| (B) | Parasite - *Camallanus lacustris* | | | |
| | **PERMANOVA** | | | |
| | explanatory variable | *d.f.* | F-value | p-value |
| Spring | **Infection (low vs. high)** | **1,161** | **1.974** | **0.089** |
| Summer | Infection (low vs. high) | 1,161 | 0.681 | 0.648 |
| Autumn | Infection (low vs. high) | 1,128 | 0.679 | 0.682 |
| (C) | Parasite - *Gyrodactylus* sp. | | | |
| | **PERMANOVA** | | | |
| | explanatory variable | *d.f.* | F-value | p-value |
| Spring | Infection (low vs. high) | 1,161 | 0.44 | 0.870 |
| Summer | Infection (low vs. high) | 1,162 | 1.44 | 0.176 |
| Autumn | **Infection (low vs. high)** | **1,126** | **2.31** | **0.023** |

**Supplementary Table 4. Results from a similarity percentage analysis (SIMPER) identify those MHC haplotypes occurring in association with either high or low parasite load of three different parasite species.** Significant results are highlighted in bold. Average contribution to overall dissimilarity (average), standard deviation (SD); average abundance per group; ordered cumulative contribution (cumsum), and permutation p-value are shown.

| (A) | Parasite - *Diplostomum* sp. | | | | | |
|---|---|---|---|---|---|---|
| **Season** | **SIMPER** | | | | | |
| Spring | MHC haplotype | average (±SD) | average (low) | average (high) | cumsum | p-value |
| | No01.No12 | 0.13 (0.14) | 0.409 | 0.351 | 0.174 | 0.427 |
| | **No15.No16** | **0.12 (0.14)** | **0.261** | **0.405** | **0.338** | **0.032** |
| | No13.N018 | 0.12 (0.14) | 0.341 | 0.311 | 0.498 | 0.678 |
| | **No05** | **0.11 (0.13)** | **0.375** | **0.135** | **0.642** | **0.017** |
| | No08.SCX15 | 0.06 (0.12) | 0.125 | 0.122 | 0.723 | 0.544 |
| | No50.No51 | 0.05 (0.11) | 0.080 | 0.135 | 0.790 | 0.175 |
| | **No47.So01.SCX03** | **0.05 (0.10)** | **0.045** | **0.149** | **0.853** | **0.022** |
| | No10.No11 | 0.04 (0.09) | 0.034 | 0.108 | 0.899 | 0.057 |
| | No07.No31 | 0.03 (0.09) | 0.057 | 0.068 | 0.940 | 0.529 |
| | No39.No40.No41 | 0.02 (0.08) | 0.080 | 0.014 | 0.971 | 0.918 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | No25.No27 | 0.01 (0.06) | 0.034 | 0.014 | 0.988 | 0.740 |
| | No36.No54 | 0.01 (0.05) | 0.023 | 0.014 | 1.000 | 0.573 |
| Spring | No01.No12 | 0.13 (0.15) | 0.346 | 0.354 | 0.169 | 0.906 |
| | No13.N018 | 0.13 (0.15) | 0.321 | 0.354 | 0.335 | 0.614 |
| | **No15.No16** | **0.12 (0.14)** | **0.198** | **0.366** | **0.484** | **0.011** |
| | **No05** | **0.10 (0.13)** | **0.333** | **0.122** | **0.614** | **0.002** |
| | **No08.SCX15** | **0.09 (0.14)** | **0.247** | **0.110** | **0.728** | **0.011** |
| | No50.No51 | 0.04 (0.10) | 0.037 | 0.122 | 0.779 | 0.077 |
| | No10.No11 | 0.04 (0.09) | 0.086 | 0.061 | 0.826 | 0.489 |
| | **No07.No31** | **0.03 (0.09)** | **0.000** | **0.122** | **0.868** | **0.005** |
| | **No36.No54** | **0.03 (0.09)** | **0.111** | **0.000** | **0.906** | **0.002** |
| | No25.No27 | 0.03 (0.08) | 0.025 | 0.085 | 0.942 | 0.156 |
| | No39.No40.No41 | 0.02 (0.08) | 0.074 | 0.012 | 0.971 | 0.057 |
| | No47.So01.SCX03 | 0.02 (0.07) | 0.012 | 0.073 | 1.000 | 0.153 |
| Spring | No13.N018 | 0.13 (0.14) | 0.431 | 0.302 | 0.169 | 0.103 |
| | No01.No12 | 0.13 (0.14) | 0.246 | 0.429 | 0.335 | 0.062 |
| | **No05** | **0.10 (0.13)** | **0.323** | **0.159** | **0.466** | **0.033** |
| | **No08.SCX15** | **0.10 (0.14)** | **0.154** | **0.286** | **0.593** | **0.046** |
| | No15.No16 | 0.09 (0.13) | 0.215 | 0.222 | 0.711 | 0.855 |
| | No50.No51 | 0.07 (0.12) | 0.169 | 0.143 | 0.802 | 0.668 |
| | **No39.No40.No41** | **0.05 (0.10)** | **0.154** | **0.032** | **0.862** | **0.031** |
| | No47.So01.SCX03 | 0.04 (0.09) | 0.108 | 0.032 | 0.907 | 0.122 |
| | No07.No31 | 0.02 (0.08) | 0.015 | 0.079 | 0.938 | 0.105 |
| | No25.No27 | 0.02 (0.08) | 0.015 | 0.079 | 0.969 | 0.103 |
| | No10.No11 | 0.02 (0.07) | 0.031 | 0.048 | 0.994 | 0.609 |
| | No36.No54 | 0.01 (0.03) | 0.015 | 0.000 | 1.000 | 0.580 |

(B) Parasite species - *Camallanus lacustris*

| **SIMPER** | | | | | | |
|---|---|---|---|---|---|---|
| Spring | MHC haplotype | average (±SD) | average (low) | average (high) | cumsum | p-value |
| | No01.No12 | 0.13 (0.14) | 0.375 | 0.378 | 0.173 | 0.741 |
| | No13.N018 | 0.13 (0.14) | 0.352 | 0.351 | 0.338 | 0.766 |
| | **No15.No16** | **0.12 (0.14)** | **0.205** | **0.365** | **0.490** | **0.016** |
| | No05 | 0.10 (0.13) | 0.284 | 0.216 | 0.623 | 0.814 |
| | No08.SCX15 | 0.07 (0.12) | 0.193 | 0.095 | 0.714 | 0.631 |
| | **No50.No51** | **0.05 (0.11)** | **0.057** | **0.162** | **0.784** | **0.026** |
| | No10.No11 | 0.04 (0.10) | 0.102 | 0.081 | 0.842 | 0.674 |
| | No47.So01.SCX03 | 0.03 (0.08) | 0.045 | 0.068 | 0.879 | 0.337 |
| | No07.No31 | 0.03 (0.08) | 0.068 | 0.041 | 0.915 | 0.765 |
| | No25.No27 | 0.03 (0.08) | 0.034 | 0.068 | 0.949 | 0.223 |
| | **No36.No54** | **0.02 (0.07)** | **0.080** | **0.000** | **0.976** | **0.049** |

| | | | | | | |
|---|---|---|---|---|---|---|
| | No39.No40.No41 | 0.02 (0.07) | 0.057 | 0.014 | 1.000 | 0.929 |

(C) Parasite species - *Gyrodactylus* sp.

| | **SIMPER** | | | | | |
|---|---|---|---|---|---|---|
| Autumn | MHC haplotype | average (±SD) | average (low) | average (high) | cumsum | p-value |
| | No13.N018 | 0.14 (0.15) | 0.344 | 0.477 | 0.182 | 0.229 |
| | No01.No12 | 0.13 (0.15) | 0.328 | 0.323 | 0.346 | 0.954 |
| | **No08.SCX15** | **0.10 (0.14)** | **0.125** | **0.292** | **0.474** | **0.027** |
| | **No05** | **0.10 (0.13)** | **0.297** | **0.154** | **0.601** | **0.037** |
| | No15.No16 | 0.09 (0.13) | 0.250 | 0.185 | 0.724 | 0.336 |
| | No50.No51 | 0.06 (0.12) | 0.172 | 0.092 | 0.806 | 0.146 |
| | No39.No40.No41 | 0.05 (0.10) | 0.109 | 0.077 | 0.865 | 0.391 |
| | No25.No27 | 0.03 (0.08) | 0.078 | 0.031 | 0.902 | 0.144 |
| | No47.So01.SCX03 | 0.03 (0.08) | 0.063 | 0.046 | 0.938 | 0.524 |
| | No07.No31 | 0.02 (0.08) | 0.047 | 0.046 | 0.968 | 0.939 |
| | No10.No11 | 0.02 (0.07) | 0.016 | 0.062 | 0.995 | 0.581 |
| | No36.No54 | 0.01 (0.03) | 0.016 | 0.000 | 1.000 | 0.130 |

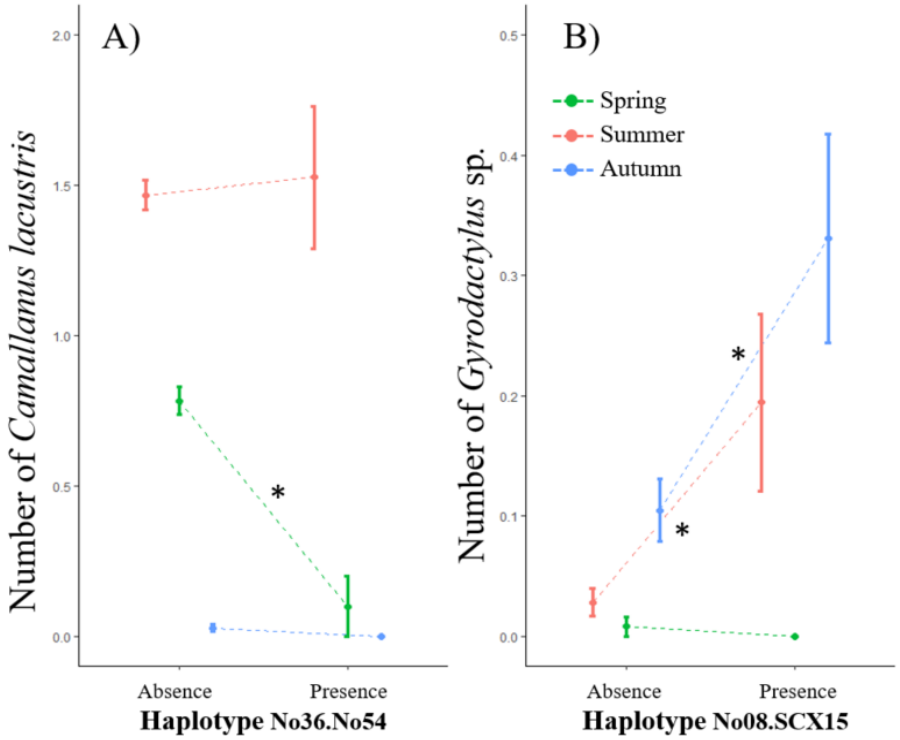
**Supplementary Table 5. Summary statistics for linear mixed effect models exploring the effect of sex, initial body condition, specific MHC haplotypes (identified from PERMANOVAs and SIMPER, see Supplementary Table 2, 3) and haplotype zygosity on infection load with three parasite species.** All models were backward selected using the *anova* function in R; Significant results are highlighted in bold. d.f. denotes degrees of freedom. 'fdr' p-values are adjusted false discovery rate correction values.

| **(A)** | **Parasite - *Diplostomum* sp.** | | | | |
|---|---|---|---|---|---|
| | **Explanatory factors** | ***d.f.*** | **F-value** | **p-value** | **'fdr' p-value** |
| **Spring** | **Haplotype No15.No15** | **1,92** | **7.577** | **0.007** | **0.014** |
| | Haplotype Zygosity | 1,83 | 0.135 | 0.714 | |
| | Initial Body Condition | 1,211 | 1.369 | 0.243 | |
| | Sex | 1,242 | 0.396 | 0.530 | |
| | **Haplotype No05** | **1,243** | **18.197** | **<0.001** | **0.001** |
| | Haplotype Zygosity | 1,241 | 1.372 | 0.243 | |
| | Initial Body Condition | 1,242 | 0.814 | 0.368 | |
| | Sex | 1,245 | 0.336 | 0.563 | |
| | Haplotype No47.So01.SCX03 | 1,119 | 1.811 | 0.181 | 0.181 |
| | Haplotype Zygosity | 1,91 | 0.025 | 0.875 | |
| | Initial Body Condition | 1,235 | 0.689 | 0.407 | |

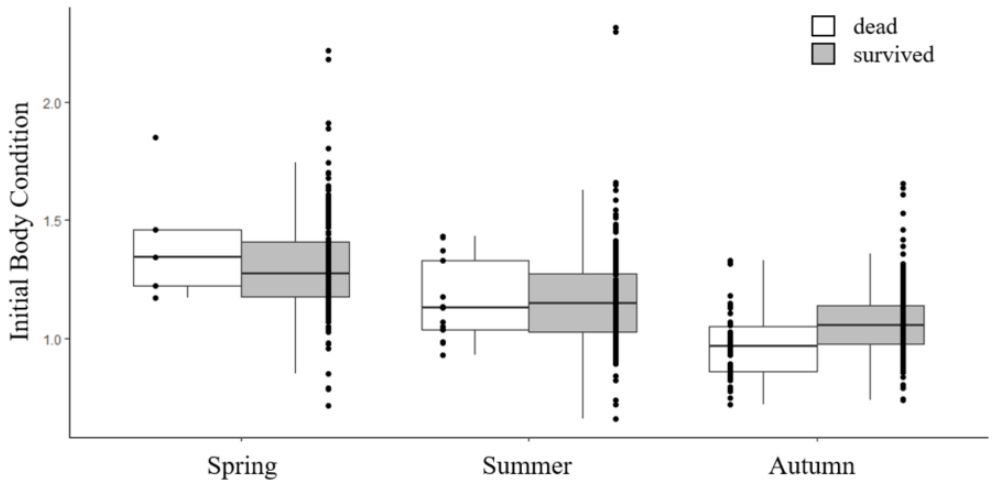
| | | | | | | |
|---|---|---|---|---|---|---|
| | | Sex | 1,240 | 0.255 | 0.614 | |
| *post results* | *autumn* | **Haplotype No39.No40.No41** | **1,89** | **6.334** | **0.014** | **0.019** |
| | | Haplotype Zygosity | 1,81 | 0.034 | 0.855 | |
| | | Initial Body Condition | 1,215 | 1.417 | 0.235 | |
| | | Sex | 1,243 | 0.214 | 0.644 | |
| **Summer** | | Haplotype No15.No16 | 1,237 | 0.315 | 0.575 | 0.575 |
| | | Haplotype Zygosity | 1,234 | 1.924 | 0.167 | |
| | | **Initial Body Condition** | **1,235** | **10.626** | **0.001** | |
| | | Sex | 1,228 | 0.017 | 0.897 | |
| | | **Haplotype No05** | **1,235** | **19.437** | **<0.001** | **<0.001** |
| | | Haplotype Zygosity | 1,235 | 0.092 | 0.761 | |
| | | **Initial Body Condition** | **1,234** | **11.287** | **0.001** | |
| | | Sex | 1,228 | 0.105 | 0.746 | |
| | | Haplotype No07.No31 | 1,235 | 1.962 | 0.163 | 0.258 |
| | | Haplotype Zygosity | 1,229 | 2.402 | 0.123 | |
| | | **Initial Body Condition** | **1,236** | **10.768** | **0.001** | |
| | | Sex | 1,227 | 0.008 | 0.928 | |
| | | Haplotype No36.No54 | 1,234 | 1.606 | 0.206 | 0.258 |
| | | Haplotype Zygosity | 1,232 | 1.781 | 0.183 | |
| | | **Initial Body Condition** | **1,235** | **10.899** | **0.001** | |
| | | Sex | 1,229 | 0.058 | 0.810 | |
| | | Haplotype No08.SCX15 | 1,228 | 2.330 | 0.128 | 0.258 |
| | | Haplotype Zygosity | 1,235 | 2.214 | 0.138 | |
| | | **Initial Body Condition** | **1,234** | **10.827** | **0.001** | |
| | | Sex | 1,227 | 0.026 | 0.872 | |
| *post results* | *autumn* | Haplotype No39.No40.No41 | 1,217 | 3.352 | 0.069 | 0.207 |
| | | Haplotype Zygosity | 1,233 | 1.690 | 0.195 | |
| | | **Initial Body Condition** | **1,236** | **9.722** | **0.002** | |
| | | Sex | 1,228 | 0.006 | 0.939 | |
| **Autumn** | | **Haplotype No05** | **1,38** | **5.256** | **0.028** | 0.056 |
| | | Haplotype Zygosity | 1,47 | 0.009 | 0.926 | |
| | | Initial Body Condition | 1,132 | 3.169 | 0.077 | |
| | | Sex | 1,174 | 0.052 | 0.820 | |
| | | Haplotype No08.SCX15 | 1,42 | 2.242 | 0.142 | 0.189 |
| | | Haplotype Zygosity | 1,72 | 0.325 | 0.571 | |
| | | Initial Body Condition | 1,177 | 1.620 | 0.205 | |
| | | Sex | 1,186 | 0.416 | 0.520 | |
| | | **Haplotype No39.No40.No41** | **1,185** | **5.774** | **0.017** | 0.056 |
| | | Haplotype Zygosity | 1,190 | 0.197 | 0.657 | |
| | | Initial Body Condition | 1,188 | 1.996 | 0.159 | |

| | | | | | |
|---|---|---|---|---|---|
| | Sex | 1,186 | 0.494 | 0.483 | |
| *post spring results* | No15.No16 | 1,144 | 0.025 | 0.874 | 0.874 |
| | Haplotype Zygosity | 1,86 | 0.242 | 0.624 | |
| | Initial Body Condition | 1,175 | 2.449 | 0.119 | |
| | Sex | 1,187 | 0.402 | 0.527 | |
| **(B )** | **Parasite species - *Camallanus lacustris*** | | | | |
| | **explanatory factors** | **d.f.** | **F-value** | **p-value** | **'fdr' p-value** |
| **Spring** | Haplotype No15.No16 | 1,78 | 3.340 | 0.071 | 0.072 |
| | Haplotype Zygosity | 1,69 | 0.518 | 0.474 | |
| | Initial Body Condition | 1,199 | 0.737 | 0.392 | |
| | Sex | 1,245 | 1.048 | 0.307 | |
| | Haplotype No50.No51 | 1,88 | 3.312 | 0.072 | 0.072 |
| | Haplotype Zygosity | 1,71 | 0.451 | 0.504 | |
| | Initial Body Condition | 1,217 | 1.219 | 0.271 | |
| | Sex | 1,244 | 1.397 | 0.238 | |
| | **Haplotype No36.No54** | **1,60** | **7.106** | **0.010** | **0.030** |
| | Haplotype Zygosity | 1,68 | 0.036 | 0.851 | |
| | Initial Body Condition | 1,203 | 0.857 | 0.356 | |
| | Sex | 1,245 | 0.931 | 0.335 | |
| **Summer** | Haplotype No36.No54 | 1,147 | 0.553 | 0.458 | NA |
| *post spring results* | Haplotype Zygosity | 1,118 | 1.099 | 0.297 | |
| | Initial Body Condition | 1,237 | 0.937 | 0.334 | |
| | Sex | 1,238 | 2.041 | 0.154 | |
| **Autumn** | Haplotype No36.No54 | 1,192 | 0.047 | 0.829 | NA |
| *post spring results* | Haplotype Zygosity | 1,192 | 0.910 | 0.341 | |
| | Initial Body Condition | 1,192 | 0.016 | 0.900 | |
| | Sex | 1,192 | 0.279 | 0.598 | |
| **(C )** | **Parasite species - *Gyrodactylus* sp.** | | | | |
| | **explanatory factors** | **d.f.** | **F-value** | **p-value** | **'fdr' p-value** |
| **Autumn** | **Haplotype No08.SCX15** | **1,43** | **6.865** | **0.012** | **0.024** |
| | Haplotype Zygosity | 1,77 | 0.021 | 0.885 | |
| | Initial Body Condition | 1,181 | 2.977 | 0.086 | |
| | Sex | 1,191 | 3.100 | 0.080 | |
| | Haplotype No05 | 1,91 | 2.890 | 0.093 | 0.093 |
| | Haplotype Zygosity | 1,96 | 0.309 | 0.579 | |
| | Initial Body Condition | 1,181 | 4.895 | 0.028 | |
| | Sex | 1,191 | 3.711 | 0.056 | |
| **Spring** | Haplotype No08.SCX15 | 1,249 | 0.198 | 0.657 | NA |
| *post autumn results* | Haplotype Zygosity | 1,249 | 0.252 | 0.616 | |
| | Initial Body Condition | 1,249 | 0.494 | 0.483 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Sex | 1,249 | 0.423 | 0.516 | |
| **Summer** | | **Haplotype No08.SCX15** | **1,238** | **17.381** | **<0.0001** | NA |
| *post results* | *autumn* | Haplotype Zygosity | 1,237 | 1.045 | 0.308 | |
| | | Initial Body Condition | 1,243 | 0.062 | 0.804 | |
| | | Sex | 1,243 | 0.050 | 0.823 | |




**Supplementary Figure 2. A) Individuals with MHC Haplotype No36.No54 had higher resistance towards *C. lacustris* (mean ±SE; square-root transformed) in spring (green), whereas B) those with Haplotype No08.SCX15 showed susceptibility towards *Gyrodactylus* sp. (mean ±SE; square-root transformed) in summer (red) and autumn (blue);**




**Supplementary Figure 3. Amongst the different seasons survival is differentially linked to initial body condition;**

**Supplementary Information – Chapter 6**

**Supplementary Table 1. SIMPER analysis on low and high abundance of a) *Diplostomum* sp. and b) *Camallanus lacustris* to identify MHC haplotypes associated with resistance to the respective parasite.** Significant results are highlighted in bold; average contribution to overall dissimilarity (average), standard deviation (sd); average abundance per group (high *Diplostomum* sp./*C. lacustris* abundance: average(high); low *Diplostomum* sp./*C. lacustris* abundance: average(low)); ordered cumulative contribution (Cumsum), permutation p-value (p) are shown.

(A) Parasite - *Diplostomum* sp.

| **SIMPER** | | | | | |
|---|---|---|---|---|---|
| MHC haplotype | average (±SD) | average (low) | average (high) | cumsum | p-value |
| No01.No12 | 0.13 (0.14) | 0.409 | 0.351 | 0.174 | 0.427 |
| **No15.No16** | **0.12 (0.14)** | **0.261** | **0.405** | **0.338** | **0.032** |
| No13.N018 | 0.12 (0.14) | 0.341 | 0.311 | 0.498 | 0.678 |
| **No05** | **0.11 (0.13)** | **0.375** | **0.135** | **0.642** | **0.017** |
| No08.SCX15 | 0.06 (0.12) | 0.125 | 0.122 | 0.723 | 0.544 |
| No50.No51 | 0.05 (0.11) | 0.080 | 0.135 | 0.790 | 0.175 |
| **No47.So01.SCX03** | **0.05 (0.10)** | **0.045** | **0.149** | **0.853** | **0.022** |
| No10.No11 | 0.04 (0.09) | 0.034 | 0.108 | 0.899 | 0.057 |
| No07.No31 | 0.03 (0.09) | 0.057 | 0.068 | 0.940 | 0.529 |
| No39.No40.No41 | 0.02 (0.08) | 0.080 | 0.014 | 0.971 | 0.918 |
| No25.No27 | 0.01 (0.06) | 0.034 | 0.014 | 0.988 | 0.740 |
| No36.No54 | 0.01 (0.05) | 0.023 | 0.014 | 1.000 | 0.573 |

(B) Parasite species - *Camallanus lacustris*

| **SIMPER** | | | | | |
|---|---|---|---|---|---|
| MHC haplotype | average (±SD) | average (low) | average (high) | cumsum | p-value |
| No01.No12 | 0.13 (0.14) | 0.375 | 0.378 | 0.173 | 0.741 |
| No13.N018 | 0.13 (0.14) | 0.352 | 0.351 | 0.338 | 0.766 |
| **No15.No16** | **0.12 (0.14)** | **0.205** | **0.365** | **0.490** | **0.016** |
| No05 | 0.10 (0.13) | 0.284 | 0.216 | 0.623 | 0.814 |
| No08.SCX15 | 0.07 (0.12) | 0.193 | 0.095 | 0.714 | 0.631 |
| **No50.No51** | **0.05 (0.11)** | **0.057** | **0.162** | **0.784** | **0.026** |
| No10.No11 | 0.04 (0.10) | 0.102 | 0.081 | 0.842 | 0.674 |
| No47.So01.SCX03 | 0.03 (0.08) | 0.045 | 0.068 | 0.879 | 0.337 |
| No07.No31 | 0.03 (0.08) | 0.068 | 0.041 | 0.915 | 0.765 |
| No25.No27 | 0.03 (0.08) | 0.034 | 0.068 | 0.949 | 0.223 |
| **No36.No54** | **0.02 (0.07)** | **0.080** | **0.000** | **0.976** | **0.049** |
| No39.No40.No41 | 0.02 (0.07) | 0.057 | 0.014 | 1.000 | 0.929 |

**Supplementary Table 2. Summary statistics for linear mixed effect models exploring the effect of sex, initial body condition, specific MHC haplotypes and haplotype zygosity.** All models were backward selected using the *anova* function in R. Significant results are highlighted in bold. d.f. denotes degrees of freedom. 'fdr' p-values are adjusted false discovery rate correction values.

| *Diplostomum* sp. | | | | |
|---|---|---|---|---|
| explanatory variables | *d.f.* | F-value | p-value | 'fdr' p-value |
| **Haplotype No15.No15** | **1,92** | **7.577** | **0.007** | **0.014** |
| Haplotype Zygosity | 1,83 | 0.135 | 0.714 | |
| Initial Body Condition | 1,211 | 1.369 | 0.243 | |
| Sex | 1,242 | 0.396 | 0.530 | |
| | | | | |
| **Haplotype No05** | **1,243** | **18.197** | **<0.0001** | **0.0004** |
| Haplotype Zygosity | 1,241 | 1.372 | 0.243 | |
| Initial Body Condition | 1,242 | 0.814 | 0.368 | |
| Sex | 1,245 | 0.336 | 0.563 | |
| | | | | |
| Haplotype No47.So01.SCX03 | 1,119 | 1.811 | 0.181 | 0.181 |
| Haplotype Zygosity | 1,91 | 0.025 | 0.875 | |
| Initial Body Condition | 1,235 | 0.689 | 0.407 | |
| Sex | 1,240 | 0.255 | 0.614 | |

| *Camallanus lacustris* | | | | |
|---|---|---|---|---|
| explanatory variables | d.f. | F-value | p-value | 'fdr' p-value |
| Haplotype No15.No16 | 1,78 | 3.340 | 0.071 | 0.072 |
| Haplotype Zygosity | 1,69 | 0.518 | 0.474 | |
| Initial Body Condition | 1,199 | 0.737 | 0.392 | |
| Sex | 1,245 | 1.048 | 0.307 | |
| | | | | |
| Haplotype No50.No51 | 1,88 | 3.312 | 0.072 | 0.072 |
| Haplotype Zygosity | 1,71 | 0.451 | 0.504 | |
| Initial Body Condition | 1,217 | 1.219 | 0.271 | |
| Sex | 1,244 | 1.397 | 0.238 | |
| | | | | |
| **Haplotype No36.No54** | **1,60** | **7.106** | **0.010** | **0.030** |
| Haplotype Zygosity | 1,68 | 0.036 | 0.851 | |
| Initial Body Condition | 1,203 | 0.857 | 0.356 | |
| Sex | 1,245 | 0.931 | 0.335 | |

**Supplementary Table 3. a) Mate choice for MHC compatibility amongst replicate populations without MHC haplotypes No05 or No36.No54. Positive results of non-random mating lead to subsequent tests to assess choice for most similar or dissimilar mates. b) Mate choice for MHC**

**compatibility in treatment groups where MHC Haplotype No05 and No36.No54 are present and comparison of choice for mates with No05 or No36.No54 to random.** Significant results are highlighted in bold. *number of choices for No05 out of a 1000.

| a) | treatment groups with random MHC haplotypes | | | |
|---|---|---|---|---|
| | mean random MHC sharing value (±SE) | mean observed MHC sharing value (±SE) | W-value | p-value |
| **Enclosure 1** | 0.44 (±0.01) | | 550690 | <**0.001** |
| Choice for MHC dissimilar? | 0.63 (±0.01) | 0.50 (±0.01) | 383080 | <**0.001** |
| Similar? | 0.30 (±0.01) | | 649600 | <**0.001** |
| **Enclosure 3** | 0.46 (±0.01) | | 534440 | **0.003** |
| Choice for MHC dissimilar? | 0.64 (±0.01) | 0.50 (±0.01) | 382200 | <**0.001** |
| Similar | 0.31 (±0.01) | | 657850 | <**0.001** |
| **Enclosure 5** | 0.43 (±0.01) | | 583240 | <**0.001** |
| Choice for MHC dissimilar? | 0.64 (±0.01) | 0.56 (±0.01) | 468690 | **0.008** |
| Similar? | 0.30 (±0.01) | | 656750 | <**0.001** |

| b) | treatment groups with resistance-associated MHC haplotype No05 | | | |
|---|---|---|---|---|
| | mean random MHC sharing value (±SE)/No05 chosen by random* | mean observed MHC sharing value (±SE)/No05 actually chosen* | W-value | p-value |
| **Enclosure 2** | 0.33 (±0.01) | 0.32 (±0.01) | 481860 | 0.113 |
| Choice for individuals with No05? | 397 | 572 | 584500 | <**0.001** |
| with No36.No54? | 217 | 203 | 476500 | 0.432 |
| **Enclosure 4** | 0.32 (±0.01) | 0.35 (±0.01) | 510700 | 0.344 |
| Choice for individuals with No05? | 409 | 603 | 597000 | <**0.001** |
| with No36.No54? | 207 | 0 | 396500 | <**0.001** |
| **Enclosure 6** | 0.38 (±0.01) | 0.42 (±0.01) | 514090 | 0.228 |
| Choice for individuals with No05? | 483 | 606 | 561500 | <**0.001** |
| with No36.No54? | 106 | 102 | 498000 | 0.769 |

**Supplementary Table 4. Summary table of generalised linear mixed effect model on proportional reproductive success acquired via sneaking behaviour for all males using treatment group, MHC haplotype No05, initial body condition and parasite load ($I_{PI}$) as explanatory variable and fish family and enclosure as random effects. Wald Chisquare test was used to obtain $x^2$ and p-values.** All models were backward selected using the *anova* function in R. Significant results are highlighted in bold. d.f. denotes degrees of freedom. *accounted for presence/absence in replicate populations.

| Proportions of eggs sneaked | *d.f.* | $x^2$ | p-value |
|---|---|---|---|
| **Treatment group** | 1,44 | 4.83 | **0.028** |
| **Individual Parasite Load** | 1,44 | 7.37 | **0.007** |
| **MHC haplotype No05*** | 1,44 | 1.44 | 0.230 |
| **Initial body condition** | 1,44 | 27.60 | **<0.001** |
| **Treatment group * Initial Body Condition** | 1,44 | 9.63 | **0.002** |

**Supplementary Table 5. Summary table of a mixed effect model on the gonadosomatic index using treatment group, parasite load ($I_{PI}$), MHC haplotype No05, and initial body condition as explanatory variables.** All models were backward selected using the *anova* function in R. Significant results are highlighted in bold. d.f. denotes degrees of freedom. *accounted for presence/absence in replicate populations.

| Gonadosomatic index | *d.f.* | F-value | p-value |
|---|---|---|---|
| **Treatment group** | 1,46 | 0.11 | 0.737 |
| **Individual Parasite Load** | 1,46 | 0.45 | 0.969 |
| **MHC haplotype No05*** | 1,46 | 4.13 | **0.048** |
| **Final body condition** | 1,46 | 0.45 | 0.504 |

**Supplementary Table 6. Summary table of a mixed effect model on the individual parasite index using treatment group, MHC haplotype No05, initial body condition and sex as explanatory variables.** All models were backward selected using the *anova* function in R. Significant results are highlighted in bold. d.f. denotes degrees of freedom. *accounted for presence/absence in replicate populations.

| Individual Parasite Load | *d.f.* | F-value | p-value |
|---|---|---|---|
| **treatment group** | 1,4 | 1.43 | 0.297 |
| **MHC haplotype No05*** | 1,22 | 1.24 | 0.277 |
| **Initial body condition corrected for sex** | 1,90 | 0.44 | 0.511 |

| | | | |
|---|---|---|---|
| **sex** | 1,86 | 0.13 | 0.717 |
| **Initial body condition * sex** | 1,95 | 3.75 | 0.056 |

**Supplementary Table 7. Results from the SIMPER analysis on low and high counts of *Diplostomum* sp. to identify coinfection with other parasite species.** Significant results are highlighted in bold. average contribution to overall dissimilarity (average), standard deviation (sd); average abundance per group (high *Diplostomum* sp. load: average(high); low *Diplostomum* sp. load: average(low)); ordered cumulative contribution (Cumsum), permutation p-value (p) are shown.

Parasite species - *Diplostomum* sp.

| **SIMPER** | | | | | |
|---|---|---|---|---|---|
| Parasite species | average (±SD) | average (low) | average (high) | cumsum | p-value |
| *Camallanus lacustris* | 0.13 (±0.12) | 3.029 | 1.972 | 0.218 | 0.757 |
| ***Apatemo*n sp.** | **0.13 (±0.11)** | **1.400** | **2.917** | **0.424** | **0.003** |
| ***Cyathocotyle prussica*** | **0.10 (±0.09)** | **0.743** | **2.222** | **0.591** | **0.001** |
| *Gyrodactylus* sp. | 0.08 (±0.09) | 1.829 | 0.889 | 0.723 | 0.916 |
| *Tylodelphis clavata* | 0.07 (±0.09) | 0.571 | 1.417 | 0.838 | 0.078 |
| *Trichodina* sp. | 0.04 (±0.05) | 1.114 | 1.417 | 0.898 | 0.223 |
| *Proteocephalus filicollis* | 0.02 (±0.03) | 0.229 | 0.222 | 0.929 | 0.750 |
| *Contracaecum* sp. | 0.02 (±0.03) | 0.286 | 0.083 | 0.958 | 0.203 |
| *Argulus foliaceus* | 0.01 (±0.03) | 0.171 | 0.167 | 0.980 | 0.960 |
| *Phyllodistomum folium* | 0.01 (±0.02) | 0.086 | 0.111 | 0.992 | 0.917 |
| *Ichthyophthirius multifiliis* | <0.01 (±0.02) | 0.057 | 0.000 | 0.996 | 0.893 |
| *Raphidascaris acus* | <0.01 (±0.01) | 0.000 | 0.028 | 0.998 | 0.130 |
| *Anguillicola crassus* | <0.01 (±0.01) | 0.000 | 0.028 | 1.000 | 0.129 |
| *Paradilepsis scolecina* | 0 | 0.000 | 0.000 | 1.000 | 1.000 |
| *Diphyllobothrium* sp. | 0 | 0.000 | 0.000 | 1.000 | 1.000 |

**Supplementary Table 8. Summary table of linear mixed effect models on final body condition retaining treatment group, MHC haplotype No05 and individual parasite load as explanatory variables after splitting by sex.** All models were backward selected using the *anova* function in R. Significant results are highlighted in bold. d.f. denotes degrees of freedom. *accounted for presence/absence in replicate populations.

| | **Final Body Condition** | ***d.f.*** | **F-value** | **p-value** |
|---|---|---|---|---|
| | **Treatment group** | 1,10 | 1.88 | 0.201 |
| ♀ | **MHC haplotype No05*** | 1,24 | 11.98 | **0.002** |
| | **Individual Parasite Load** | 1,50 | 0.39 | 0.538 |
| | **Treatment group** | 1,31 | 0.35 | 0.558 |

| | | | | |
|---|---|---|---|---|
| ♂ | **MHC haplotype No05*** | 1,51 | 1.56 | 0.217 |
| | **Individual Parasite Load** | 1,43 | 6.82 | **0.012** |
| | **MHC haplotype No05 * Individual Parasite Load** | 1,43 | 8.26 | **0.006** |

**Supplementary Information – Chapter 7**

**Supplementary Table 1. Results from the SIMPER analysis comparing prey abundances across enclosures with and without individuals carrying a resistance associated MHC haplotype and comparing that to control measurements taken from outside the enclosures.** Significant results are highlighted in bold. average contribution to overall dissimilarity (average), standard deviation (sd); average abundance per group (replicate populations with resistance associated MHC haplotype: averageR; replicate populations without resistance associated MHC haplotype: averageS; measures taken from outside the enclosures: averageC); ordered cumulative contribution (Cumsum), permutation p-value (p) are shown.

| **Prey Family** | **average (±SD)** | **averageR** | **averageC** | **cumsum** | **p-value** |
|---|---|---|---|---|---|
| ***Cyclopoida*** | 0.18 (±0.14) | 0.568 | 1.244 | 0.347 | **0.023** |
| ***Bosminidae*** | 0.16 (±0.15) | 1.023 | 1.707 | 0.651 | 0.578 |
| ***Daphniidae*** | 0.11 (±0.12) | 0.432 | 0.585 | 0.858 | 0.938 |
| ***Calanoida*** | 0.07 (±0.10) | 0.136 | 0.439 | 1.000 | 0.412 |

| | **average (±SD)** | **averageR** | **averageS** | **cumsum** | **p-value** |
|---|---|---|---|---|---|
| ***Cyclopoida*** | 0.16 (±0.18) | 0.568 | 0.476 | 0.314 | 0.743 |
| ***Bosminidae*** | 0.16 (±0.18) | 1.023 | 0.976 | 0.619 | 0.572 |
| ***Daphniidae*** | 0.13 (±0.15) | 0.432 | 0.476 | 0.872 | **0.026** |
| ***Calanoida*** | 0.07 (±0.12) | 0.136 | 0.238 | 1.000 | 0.771 |

| | **average (±SD)** | **averageC** | **averageS** | **cumsum** | **p-value** |
|---|---|---|---|---|---|
| ***Cyclopoida*** | 0.18 (±0.14) | 1.244 | 0.476 | 0.329 | **0.028** |
| ***Bosminidae*** | 0.18 (±0.16) | 1.707 | 0.976 | 0.648 | 0.069 |
| ***Daphniidae*** | 0.11 (±0.12) | 0.585 | 0.476 | 0.850 | 0.834 |
| ***Calanoida*** | 0.08 (±0.11) | 0.439 | 0.238 | 1.000 | 0.083 |